NASA TECHNICAL TRANSLATION NASA TT F-15849

# PROBLEMS OF SPACE BIOLOGY, VOL. 27.

# RADIOBIOLOGY AND GENETICS OF ARABIDOPSIS

V. I. Ivanov

Translation of: "Problemy Kosmicheskoy Biologii, Tom. 27. Radiobiologiya i Genetika Arabidopsisa, Moscow, "Nauka" Press, 1974, pp. 1-191

(NASA-TT-F-15849) PROBLEMS OF SPACE BIOLOGY. VOLUME 27: RADIOBIOLOGY AND GENETICS OF ARABIDOPSIS (Kanner (Leo) Associates) 192 p HC $7.00 CSCL 06C

N75-10679

Unclas
G3/51 51132


NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, DC 20546 OCTOBER 1974

STANDARD TITLE PAGE

| 1. Report No. | 2. Government Accession No. | 3. Recipient's Catalog No. |
|---|---|---|
| NASA TT F-15,849 | | |

| 4. Title and Subtitle | 5. Report Date |
|---|---|
| Problems of Space Biology. Vol. 27. Radiobiology and Genetics of Arabidopsis | September 1974 |
| | 6. Performing Organization Code |

| 7. Author(s) | 8. Performing Organization Report No. |
|---|---|
| V. I. Ivanov | 10. Work Unit No. |
| | 11. Contract or Grant No. NASw-2481 |

| 9. Performing Organization Name and Address | 13. Type of Report and Period Covered |
|---|---|
| Leo Kanner Associates<br>Redwood City, California 94063 | Translation |

| 12. Sponsoring Agency Name and Address | 14. Sponsoring Agency Code |
|---|---|
| National Aeronautics and Space Administration, Washington, D. C. 20546 | |

15. Supplementary Notes

Translation of Problemy Kosmicheskoy Biologii, Tom 27. Radiobiologiya i Genetika Arabidopsisa, Moscow, "Nauka" Press, 1974, pp 1-191

16. Abstract

Arabidopsis thaliana is discussed as an optimum object of aerospace research on radiobiology, radiation genetics and general botanical research. Varied aspects of plant research are considered: survival, growth, development, fertility, effects of irradiation, sexual and asexual reproduction under zero gravity. The importance of the abundance of arabidopsis mutants and their small size are cited as some of the important merits of this plant's use as an object of space research.

| 17. Key Words (Selected by Author(s)) | 18. Distribution Statement |
|---|---|
| | Unclassified-Unlimited |

| 19. Security Classif. (of this report) | 20. Security Classif. (of this page) | 21. No. of Pages | 22. Price |
|---|---|---|---|
| Unclassified | Unclassified | | |

NASA-HQ

TABLE OF CONTENTS

# RADIOBIOLOGY AND GENETICS OF ARABIDOPSIS

V. I. Ivanov

## Introduction /5*

The search for and incorporation of new objects of research in the natural sciences has usually been governed by the emergence of scientific problems whose solution is faced with an unsufficient prior understanding of the object. So it was at the dawn of evolution of modern genetics to intensively study the basic phenomena of inheritance it was necessary to rapidly and easily multiply under laboratory conditions a biologic form possessing a large complex of hereditary features suited and convenient for precise and objective quantitative calculation. At that time, T. Kh. Morgan and his followers had begun vast studies on the genetics of drosophila; as is well known, this led to the discovery after some 10-15 years of the chromosomic theory of inheritance--one of the basic concepts of all modern genetics and biology. Later on, already into our period, rapid and significant progress in genetics on a new (molecular) level was made due to research on bacteria, viruses and phags--objects more suited for complex study of the molecular foundations of inheritance and variation using genetic and biochemical methods. Similar examples can also be cited, but many of them are well known and these two are sufficient to illustrate the confirmed value of selecting appropriate objects to solve problems of biology. The aforesaid is also completely valid with respect to research in aerospace biology, since in that case the specifications of conditions of bodily viability in the circumstance of space flight can give the successful choice of test objects a primary decisive value.

The incorporation of new objects into biologic research is substantial in yet another respect: in addition to solving actual problems for whose study these objects were initially selected, the study of new objects expands and reinforces the factual base on which is constructed the entire set of notions on phenomena of life. The last fact is governed by the fact that only a comparative study of different forms of living organisms permits us to separate out effects inherent to all of diverse living nature or /6 its individual large-scale subdivisions from the enormous multitude of distinctive features intrinsic only to certain forms or even to isolated individuals. Indeed (if we again refer to examples from the realm of genetics) even the basic findings of G. Mendel's experiments sort of remained the "pea laws", as they were at one time ironically called due to misunderstanding, while the

* Numbers in the margin indicate pagination in the foreign text.

chromosome theory of inheritance sort of remained the theory of the genetics of drosophila, if their primary positions and consequences were not to be repeatedly verified and confirmed in many members of all realms of living nature. As concerns the achievements of molecular genetics, namely today in world biology there is intense experimental verification of the fact that from its positions, some things relate to the field of molecular genetics of microrganism protocaryotes, and some--to the field of general molecular genetics. Here, however, one of the obligatory prerequisites of rapid progress is the selection of objects most suitable for study using the methods of this branch of biology.

This is all related--the value of new objects and the comparative study of different biologic forms--to any branch of biology, including aerospace biology, and likewise to radiation genetics and radiobiology, some of the problems of which will be discussed in this book.

Beginning in the 1950's, in world literature on experimental biology, particularly in genetics, there began to appear separate studies carried out on the plant Arabidopsis of the family of cruciferae--Arabidopsis thaliana (L.) Heynh. The provocation for conducting these studies was an article by the German botanist F. Laibach "Arabidopsis thalian (l.) Heynh als Object für genetische und entwicklungsphysiologische Untersuchungen" (Laibach, 1943) in which the author, generalizing his almost forty-year study of arabidopsis, convincingly proved that ephemeral races of this little plant have a large number of certain advantages for conducting experimental biologic research on the most diverse planes (Ch. I and II).

We must say that experimental botanists (primarily geneticists) have long sought ephemeral plants an object comparable in its merits to the classic genetic object--drosophila. Thus, here in the USSR in 1921, B. M. Kozo-Polyanskiy planned toward this end a special expedition into the Lake Elton region to collect local flora of the ephemerae and study their biology and karyology. The findings of this expedition were published in N. N. Titova's article "In search of botanical drosophila"(1935). After comparing about two dozen species, the author concluded that the most suitable pretender for the name "botanical drosophila" was Arabidopsis thaliana (L.) Heynh. But the work of Titova virtually went unnoticed and it took the ap-/7 pearance of Laibach's article and the first studies of E. Reinholz on radiation mutagenesis in arabidopsis (Reinholz, 1947, 1954) and the studies of G. Langridge on biochemical genetics of this plant based on mass aseptic culture (Langridge, 1955, 1957) to have the incorporation of arabidopsis into laboratory practice accepted in many countries. Already a large (about 800) and ever-increasing number of publications indicate that this plant has not betrayed the hopes invested in it. Experimental research of arabidopsis was begun even here in the USSR. A pioneer of this research was

the Department of Genetics and Selection of Leningrad University (Kvitko, 1960; Kvitko and Myuller, 1961) under whose direct influence research on arabidopsis was begun at the Institute of Plant Physiology and Biophysics of the Tadzhik Academy of Sciences in Dushanbe and (upon the initiative of N. V. Timofeyev-Resovskiy) at the Institute of Medical Radiology of the USSR Academy of Sciences in Obninsk. At the present time, new focal points of 'arabidopsology' have emerged in Moscow, Leningrad, Novosibirsk, Perm' and Minsk; and there are almost a hundred Soviet publications on this subject.

A general conclusion drawn from the research done on arabidopsis is the undoubted promise of this object in the most diverse biologic disciplines.

Particularly many studies have been done on the genetics and radiobiology of arabidopsis. In these studies, methods of experimental work with arabidopsis have been developed and verified. Of late, arabidopsis has begun to be used as the object of aerospace research.

But arabidopsis, as every new object of study, in revealing new possibilities simultaneously creates certain problems; these are primarily the result of its feeble investigation. In particular, the necessary requisite for detailed radiation-genetic research of arabidopsis is a sufficiently precise and complete radiobiologic characterization of this plant. There is no integral characterization of arabidopsis in world literature, and its composite elements have been spread in roughly a hundred journal articles and comments. Consequently, one of the problems of this book is to generalize and analyze original and literature data on the basic somatic and genetic effects of irradation in arabidopsis. (Here and furthermore the term "somatic effects of irradiation" denotes radiobiologic reactions of the plants appearing in the same diploid generation, whose initial phase--the embryo of a mature seed--is subjeted to radiation effects, namely: death of the plants, depression of their growth and evolution, reduction of fertility).

Aside from the fact that the parallel study of somatic and genetic effects of radiation assists in the expansion and deepening of knowledge on arabidopsis as an object of experimental biology, this study (as has been shown in the concluding sections of this book) has an even more general meaning: it promotes a better understanding of the nature of relationships between radiation effects developing on the cellular level (genetic effects) and effects realized on the level of the overall multicellular plant organism (somatic effects). /8

Since radiation is one of the main factors of space flight, a monographic description of radiobiology and radiation genetics

of arabidopsis as a promising object of aerospace research is of definite interest to aerospace biology. Indeed, as we said before, in genetic and radiobiologic research the basic methods of experimentation on arabidopsis have been developed, and the knowledge of these methods is a necessary prerequisite for successful application of this new object.

In view of the fact that this book is the first Soviet monograph on arabidopsis it contains--in addition to the basic theme of radiobiology and genetics of this plant--a general characterization of arabidopsis and methods of its cultivation and study are examined.

# PART I

# ARABIDOPSIS THALIANA (L.) HEYNH.

# AS AN OBJECT

# OF BIOLOGIC RESEARCH STUDIES

General information on arabidopsis as an object of experimental biology can be found in several reviews (Laibach, 1943, 1965; Kvitkov, Myuller, 1961; Kribben, 1964; Reinholz, 1965; Ivanov et al., 1966; Postlethwait, Enochs, 1967; Redei, 1969). But due to the rapid development of arabidopsis research, some of these reviews already require supplementation with new data; other surveys are devoted more or less to particular problems. Thus it appears advisable to preface the data on radiobiology and radiation genetics of arabidopsis with a botanical characterization of this species (Chapter 1) and also to summarize the existing literature data on arabidopsis as an object of biologic (primarily genetic) research and the main trends of study of this plant in genetics (Chapter 2) and aerospace biology (Chapter 3). As concerns radiobiology of arabidopsis, the second and third parts of this book are devoted to it in particular. /9

## Chapter 1. Botanical Characteristics of Arabidopsis thaliana (L.) Heynh.

### 1.1. The Family Arabidopsis Heynh.

Modern classification of plants (Takhtadzhyan, 1966) relates /10
Arabidopsis thaliana (L.) Heynh. to the subtribe Arabidinae of the tribe [illegible]..bideae (Hayek, 1908) of the family Cruciferae A. L. de Jussieu (Brassicaceae Burnett, 1835) of the order Capparales of the class Mag[..]atae(Dicotyledones) of the branch Magnoliophyta (Angiospermae). But this classification of A. thaliana can hardly be considered firmly established with respect to the lower [..], since modern classification of the family Cruciferae (Brassicaceae) still contains a number of serious contradictions [..] the boundaries of families and the taxonomic independence of several of them (Freyn, 1889; Vandendries, 1909; Bo[..]tsev, 1957, 1959; Scholz, 1962; Berger, 1965; Zyablitskaya, 1969, 1972). The complex and confused history of the family Arabidopsis is clearly illustrated by the large number of synonyms in other previously described species of A. thaliana , namely: Arabis thaliana L., Sisym[..]m thalianum Gay, Stenophragma thalianum Celak., Erysi[..] thalianum Kittel. The origin of these synonyms is associated with repeated revisions of the familiy Cruciferae (De Candolle, 1925; Prantl, 1891; Hayek, 1908) following its initial description by K. Linnaeus (1737).

Besides A. thaliana, the family Arabididopsis contains in its modern classification 12 species: A. himalaica Schulz, A. kneukeri Schulz, [...]korshinskii Botsch., A. lasiocarpa Schulz, A. mollissima Busch, [...]parvula Schulz, A. pumila Busch, A. stricta Busch, A. suecica [..], A. toxophylla Busch, A. verna (C. Koch) Busch, A. wallichii Busch (1939; Schulz, 1936; Bochantsev, 1957, 1959; Berger, 1965).

Photographs of herbal specimens of several species of arabidopsis are illustrated in Fig. 1. They are all herbaceous, one- two- and perennial plants having a near-root rosette of leaves, one or more simple or branching leafy or leafless stalks crowned with racemes in the form of a simple cluster of white or yellow flowers of a regular cruciferous structure; the fruit is a pod. The areal of the family--moderate points of a [...]hemisphere. Of the thirteen species of Arabidopsis, seven grow in the USSR: A. korshinskii, A. mollissima, A. parvula, A. pumila, A. thaliana, A. toxophylla and A. wallichii (Busch, 1909, 1939; Vasil'chenko, 1948; Pasyaukova, 1951; Grigoryev, 1953; Bochantsev, Vvedenskiy, 1955; Nikitina, 1955; Vasilyeva, 1961; Zakirov, 1961; Ikonnikov, 1963; Bochantsev, 1965; Yunusov et al., 1969). Most of the spec- /12
ies of this family grow in the Middle and extratropical part of the Himalayas (Busch, 1939; Parsa, 1951; [..]amura, 1960; Bochantsev, 1965; Ratcliffe, 1965). The species of the family Arabidopsis


Fig. 1. Herbal specimens of A. korshinskii Botsch.(A), A. pumila (Steph.) Bush (B) and A. wallichii (Y. Hook) Busch (C).

are generally xerophytes; some are halophytes; others occur in rocky areas or in rocks.

In kariosystematic respects, the family Arabidopsis has been studied quite poorly. It was found that in A. thaliana 2n = 10 (Laibach, 19..7; Winge, 1925; Jaretzki, 1928, 1932; Manton, 1932; Boecher, ...rsen, 1958; Löve, Löve, 1961; Ginter, Ivanov, 1968) and that three ...--A. wallichii, A. pumila (A. griffitiana) and A. korshinski form a series with 2n = 16, 32 and 48, respectively (Ginter, Ivanov, 1968). The number of chromosomes in other species of the family Arabidopsis has not been established.

To explain the taxonomic relationships among the family Arabidopsis and its interrelationships with closely related plants, research has recently begun on inter-species and inter-generic hybrids (Laibach, 1958; Kribben, 1965; Berger, 1966, 1968, 1968a, 1968b; Redei, 1972), methods of numerical taxonomy (Crovello, 1968; Zyablitskaya, 1969, 1972; Fershtat et al. 1971) and comparative study of variation of different taxons (Li et al., 1967; Fershtat et al., 1971), which permits us to hope for progress in the field of microsystematics of arabidopsis in the near future.

## 1.2. The species A. thaliana (L.) Heynh.

For experimental research, the most interesting species of Family Arabidopsis is A. thaliana. It is a small annual, less often biennial leafy plant. Its botanical description according to N. A. Busch (1939) appears so: the stalk is generally a single, 4.5-7.0 cm high, thin, straight stalk; [...] or branching, covered with simple or 2-3 leaves; the leaves are elongated lanceolates and elongated with remote teeth; root leaves are collected into a rosette and are tapered into a short [...]; the cluster is compressed in flowering, and then is greatly elongated and fragile, 8-40 flowers; sepals are 1.5-2 mm long, elongated, dull; petals are white, 3-4 mm long, elongated; the lateral honey glands are hemispherical, quite large; ovary has 48-68 seed-buds; pedicles in the fruit are thin, protuberant, 4-15 mm long; pods are [...] on top, bare, often bent, 9-18 mm, rarely up to 30 mm long, 0.75 mm wide; the style is thin, short; leaves have long thin vein; partition is transparent, brilliant, without [..]; seeds are reddish-brown, oviform, homogeneous, 0.5 X 0.4 X 0.3 mm.

The areal of A. thaliana encompasses almost the entire Palearctic: from Sweden in the north to North Africa in the south; from Japan in the east to Iceland. This species is also known in North/13 America and Australia. The A. thalian is adapted in North America on the Atlantic and Pacific coastlines and is absent from the central regions; data on it in early floral descriptions of the coas-

Fig. 2. Herbal specimens of A. thaliana (L.) Heynh. of different geographic origin.
A) Moscow region; B) Leningrad region; C, D) Tadzhik SSR.

/14

tal areas leads most botanists to believe that A. thaliana was accidentally and quite recently introduced to America from Europe and Asia (Redei, 1969, 1970). Nothing is yet known about the origin of this plant in Australia.

A typical habitation of A. thaliana is light, well-heated sections with light sand, sometimes rocky soil and, usually, poorly developed vegetation. These may be timber cuts, road cuts, garbage dumps, etc.

Within the vast species areal of A. thaliana there are many sub-species, varieties and races (Ratcliffe, 1961; Adams, 1964; Cetl, 1965a; Effmertova, 1967; Loginov et al., 1972). The world collection of A. thaliana races created by Laibach and currently being maintained in Goettingen University counts over 100 forms (Roebbelen, 1965e). Common to all these forms is the fact that they are all plants of long day. Distinctions between them are both in their morphology, particularly in ecology and physiology. Among them there are winter and spring, early and later forms which also differ in the duration of the embryonic diapause--from its virtual absence to two or more months (Haerer, 1951; Laibach, 1951; Effmertova, Cetl, 1966; Kucera, Cetl, 1967). Most forms differ in temperature conditions required for their development (Langridge, 1963, 1963a; Cetl et al., 1967; Ashraf, 1970a, 1971, 1971a) as well as in other physiologic, especially photosynthetic properties (Tauböck, 1943; Kasyanenko, 1964; Usmanov et al., 1970). Herbal forms of several natural forms of A. thaliana are shown in Fig. 2.

The high intra-species polymorphism of A. thaliana with respect to physiologic features served as Laibach's main cause to recommend this species as the most suited object for research on the physiology of development of plants. And in reality, within a comparatively short period of time (virtually within the post-war period) in research on arabidopsis it has been possible to produce important data on the physiologic bases of the process of yarovization. (Napp-Zinn, 1953, 1954; Laibach, Zenker, 1954; Barendse, 1963), photoperiodism effects (Gregory, Hussey, 1953; Brown, 1969), processes linked with conversion of plants to blossoming (Laibach, 1940, 1943a, 1949; Clauss, Rauch, 1956; Napp-Zinn, 1960; Brown, Klein, 1971; Kranz, 1971, 1971a, 1971b; Tsuboi, Ianagishima 1971) and also in other fields of physiology of development. The fact that arabidopsis is at the same time a suitable object of genetic research granted the explanation of the genetic bases of the physiologic processes being studied (Napp-Zinn, 1955, 1963). Thus, for example, it was found that inter-racial distinctions in the duration of vegetation period can be governed by mutations of a small number (2-3) of interacting genes (Laibach, 1943a; Napp-Zinn 1965; Van der Veen, 1965) or even mutations of discrete genes (Hä- /15

rer, 1951; Dierks, 1958; Napp-Zinn, 1963) accompanied by a set of genetic modifiers (Dierks, 1958).

But the physiology of development has not yet become the main field of application of arabidopsis in experimental research. Both in a number of publications and in the breadth of scope of problems, the leading focus in this respect belongs to genetics, especially to research on induced mutation processes. We could believe, however, that as genetic study of arabidopsis broadens its merits as an object of other fields of experimental biology (terrestrial and aerospace) will increase abruptly.

## Chapter 2. Genetics of A. Thaliana (L.) Heynh.

This chapter will briefly examine the primary merits of arabidopsis as an object of genetic research; the main types of mutations known in this plant are described and a review of the main trends in genetic research conducted on this object is given.

### 2.1. A. thaliana as an object of genetic research

One of the factors inclining geneticists to this plant was the presence in A. thaliana of an entire series of ephemeral races having a very short life cycle (about one month) and the virtual absence of an expressed embryonic diapause. The most popular fast growing natural races of arabidopsis are Dijon (Di), Enkheim-1 (En-1), Estland (Est), Limburg (Li) and Landsberg (La). For laboratory research several selection lines are also isolated from these races. They are all very similar to each other in morphology and physiology, so that the choice of any one of them as an object of study often is subjective in nature.

For genetic experiments, another feature of arabidopsis is /16
also important, namely, the high rate of seed production. Even under minimum conditions of a test-tube culture (Ch. 4) from one plant we can yield about 200 seeds; and using special methods of cultivation (sparse sowing, abundant nutrition, short day with bright illumination for first 20-30 days of development) the number of seeds in one plant may be brought up to 10,000-40,000 (Ivanov et al., 1966; Feenstra, 1967).

Self-pollination occurring in arabidopsis normally still before dehiscence is suitable for detecting emergent mutations and for rapidly isolating them into pure lines (Mueller, 1961). At one time, true, based on the wide variability of quantitative features in lines of arabidopsis isolated from natural populations, it was believed that cross pollination can have substantial value

in reproduction of arabidopsis in nature (Napp-Zinn, 1964; Dobrovolna, 1967; Jones, 1968; Karbe, Roebbelen, 1968). But in special tests on combined cultivation (with dense packing of plants) of a mixture of genetically marked lines--non-dropped Wilna-2 and dropped Langridge (Lawrence, Snape, 1971)--it was found that the portion of offspring produced as a result of cross pollination, even under the favorable conditions created especially for this test was only about 1%.

Similar data on the very low frequency of spontaneous cross pollination were also derived in combined cultivation of plants of the race En-1 and derived from this race double mutants hairless, yellow seeds (Roebbelen, 1971). At the same time, arabidopsis quite easily yields to artificial cross pollination both with the employment of flower castration (Mueller, 1961; Feenstra, 1965) and without it (Usmanov, Myuller, 1970). In the latter case, in order to avoid contamination of the test offspring by seeds originating in self-pollination or spontaneous cross pollination, the parental forms must be appropriately chosen: maternal form must have a heterostyle with advanced development of the female organs (indeed, this is precisely what is seen in some natural races of arabidopsis, e.g., En-1), while the paternal form must have recessive markers (Ivanov et al., 1966; Barabas, Redei, 1971).

It is also important for geneticists that the number of chromosomes in A. thaliana 2n = 10; consequently, this species has five groups of coupling, which greatly simplifies genetic analysis. True, the chromosomes in arabidopsis are small: the metaphase length of the largest of them is under 3 microns, three pairs of point chromosome and one intermediate in length. Nonetheless, after modifying the methods of preparation of the compounds (Steinitz-Sears, 1962, 1963, 1966), cytologists were able to study in arabidopsis mitosis and meiosis in ordinary diploids (Steinitz-Sears, 1962, 1963, 1964; Polyakova, 1964) and also /17
work out karyotin polyploidals (Wricke, 1955; Bronkers, 1963; Bouharmont, 1965, 1966, 1967) and aneuploidal forms (Arnold, 1964; Arnold, Cruse, 1965; Roebbelen, Kribben, 1966; Steinitz-Sears, Lee-Chen, 1968, 1970; Lee-Chen, Steinitz-Sears, 1969; Bouharmont, 1969).

## 2.2. Mutations of A. thaliana

Let us now examine the primary business of geneticists--mutations. As in any living organism, mutation variability in arabidopsis embraces the entire spectrum of large and small, quality and quantity, morphologic, physiologic and biochemical features and properties. Some classes of mutations encountered in arabi-

dopsis are very suitable for research in different fields of genetics.

The first mutations found in arabidopsis were those affecting the growth of the plants (yarovization, photoperiodism) about which we talked earlier and whose discovery was a by-product of physiologic tests. But even in 1947, E. Reinholz carried out special tests aimed at obtaining mutations in arabidopsis after X-ray irradiation of the seed. In these tests she isolated about 20 different mutant forms characterized mainly by variations in shape of the rosette leaves (Fig. 3). In subsequent research, Reinholz and other authors obtained many morphologic mutations affecting both the shape and structure of individual plant organs and their overall form (McKelvie, 1962; Redei, Hirono, 1964; Redei, 1967; Arnold, 1965; Roebbelen, 1965d; Reinholz, 1966; Nikolov, 1968; Usmanov, 1970, and others). Particular large lists of morphologic mutations appears in the works of A. D. McKelvie--about 200; Ch. V. Nikolov --over 80 and in the series of studies of Redei et al.--over 50. The total number of morphologic mutations described in arabidopsis already exceeds 400. As usual in plants, many morphologic mutations have pleiotropic effects with respect to viability (Redei, Steinitz--Sears, 1961; Redei, 1962b; Buggert, Roebbelen, 1970); with respect to periods of development (Hussein, Van der Veen, 1965; Van der Veen, 1965a; Cetl, 1966; Kucera, 1968), sensitivity to temperature (Brown, 1964; Corcos, 1969) and so forth.

A particular group of plants are the so-called chlorophyllic or (more broadly) pigment mutations which determine change in normal coloration of green parts of the plants to white, yellow, pale and/or yellowish-green, intense-green, etc. Among the chlorophyllic mutations we know of those which bring about a rise or drop in viablity, have no effect on it or even have a lethal effect in the very earliest stages of ontogeny. Disturbances of pigmenta- /18
tion can be constant (for the entire life cycle) or change in time to a certain direction. All these types of chlorophyllic mutations are known in arabidopsis, and the Lamprecht system (1960, 1965) is used to classify them. As with other plants, the high frequency of encounterability and eas of diagnosis of chlorophyllic mutations makes them an extremely popular plant in research on mutagenics; the key value of chlorophyl in the life of the plants governs the increased interest which geneticists and botanists have in the phenogenetics of chlorophyllic mutations. The total number of publications on chlorophyllic mutations of arabidopsis is already quite large and it is hardly feasible to discuss all of them in detail. But it is still worth a brief look at the most inter- /19
esting findings.

Mutations of the type chlorina and mutations like it with defective pigmentation and with normal or reduced viability are the best studied in arabidopsis. It was found that pigment mutations

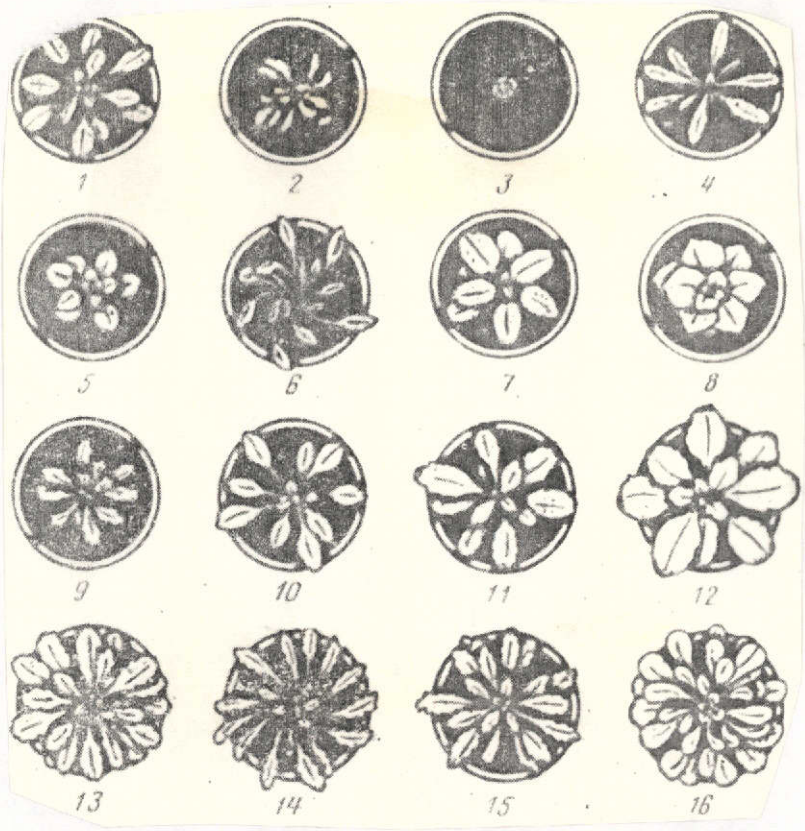



Fig. 3. First Morphologic Mutations of Arabidopsis Obtained as a Result of X-ray Irradiation of seeds of the race En (Reinholz, 1947): 1) En., 2) Mut. procera; 3) Mut. rosula; 4) Mut. dentata; 5) Mut. cordifolia; 6) Mut. tortifolia; 7) Mut. sessilia; 8) Mut. latissima; 9) Mut. undulata; 10) Mut. glabra; 11) Mut. lata; 12) Mut. latifolia; 13) Mut. grandifolia; 14) Mut. grandiserrata; 15) Mut. tarda; 16) Mut. tardeflorenz.

form a complex group including both mutations of nuclear genes (Roebbelen, 1957; Kasyanenko, Timofeyev-Resovskiy, 1967) and cytoplasmic mutations (Roebbelen, 1962, 1964, 1965). Both may have different effects on the photosynthetic apparatus of the plants: to change the content of different forms of chlorophyl (Hirono, Redei, 1963a, Hirono, 1964; Kasyanenko, Nasyrov, 1968a; Kasyanenko, Usmanov, 1969; Giller et al., 1971; Kranz, 1972) or to lead to their complete absence (Roebbelen, 1957a). There is a sharp positive correlation between the degree of disturbance of the ultrastructure of the chloroplasts and a reduction of the synthesis of chlorophyl (Veleminsky, Roebbelen, 1965, 1966). It is natural that disturbances in the ultrastructure and the content of pigments in the chloroplasts leads to total series of other

indirect disturbances in the functioning of the photosynthetic apparatus of mutant plants (Nasyrov, Kasyanenko, 1965; Kasyanenko, Giller, 1967; Kasyanenko, Nasyrov, 1968; Kasyanenko et al., 1971; Svachulova, 1971). It is also extremely interesting that both the appearance of cytoplasmic chlorophyllic mutations and the mutability itself of the plastome are under the control of the nuclear genes (Roebbelen, 1964a, 1966a, 1966b).

By the nature of action and the phenotypic appearance of chlorophyllic mutations, they may be considered both morphologic and biochemical. But in arabidopsis we know of a certain class of hereditary changes which can be called biochemical mutations in the narrow meaning of the word. These are auxotrophic mutations which manifest a mutant phenotype or are non-viable in cultivation with an ordinary mineral medium and normalize in cultivation in a medium with addition of a metabolite which the specific mutant lacks (Fig. 4).

Prior to the introduction of arabidopsis into genetic research, biochemical mutations could only be obtained experimentally in microorganisms. When Langridge developed methods of mass aseptic culture for arabidopsis in purely mineral media (Langridge, 1957), and several authors proposed effective methods of detecting biochemical mutations (Langridge, 1958; Hirono, Redei, 1964; Jacobs, 1964c, 1965, 1965a; Feenstra, 1966; Li, Redei, 1968), it first become possible to easily obtain biochemical mutations in the higher plants. The first biochemical mutation in arabidopsis (deficient in thiamine) was described by Langridge (1955). In later years the production of biochemical mutations was taken up in many laboratories (Jacobs, 1964b; Feenstra, 1965a; Redei, 1964d; Redei, Barabas, 1971; Langrdige, 1965; Li, Redei, 1967, 1968; Roebbelen, 1968; Abdulayev, et al., 1970; Kasyanenko, Nasyrov, 1970; Kasyanenko et al, 1971). Thus by now several dozens of biochemical mutations of this plant have been described. And here is what is remarkable: the overwhelming majority of these mutations are auxotrophic either in thiamine or in one of its predecessors in the biosynthesis paths of pyrimidine or thiazole. Mutations auxotrophic in other metabolites are encountered quite rarely. They relate to specific mutations whose phenotypes become normal in cultivation in media with glucose, saccharase, leucine, as well as in "complete" media with additives of vegetable or yeast extracts. In this regard, the higher plants sharply differ from microorganisms which have biochemical auxotrophic mutations which are readily produced in the most diverse metabolites. It is possible that the problem of producing auxotrophic mutations in the higher plants is caused by their complete auxotrophicity and probably, by the presence of 'reserve' paths of biosynthesis of the majority of necessary substances.

/20

/21

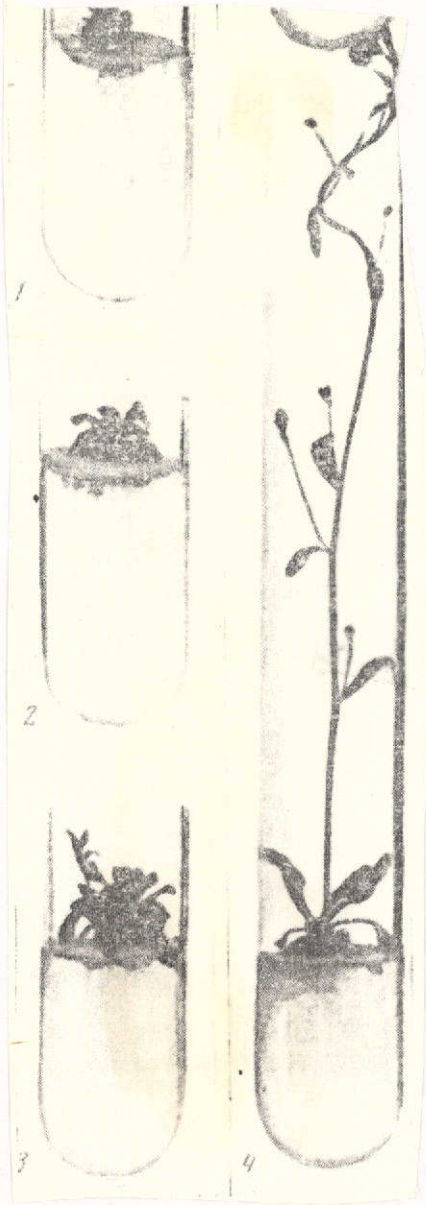


Fig. 4. One of the first biochemical mutations of Arabidopsis. Mutant phenotype (1) is normalized in increasing concentrations of saccharose; 2) 20 mg per plant; 3) 50 mg; 4) 100 mg (Langridge, 1958a).

Consequently, it is no surprise that biochemical genetics of arabidopsis has concentrated on the reconstruction of the means of biosynthesis of thiamine and analysis of the genetic control of this biosynthesis. As a result of wide research in this direction, the means of thiamine biosynthesis were made clear (Redei, 1960, 1962, 1962a; Feenstra, 1965; Li, Redei, 1969a) and it was also found that there are series of complementary and non-complementary allelas in the corresponding locuses of the chromosomes (Feenstra, 1964, 1965b; Van den Berg, Feenstra, 1968; etc.). More detailed information on this and other branches of biochemical genetics of arabidopsis can be found in Redei's survey (1969).

It was mentioned above that some chlorophyllic and biochemical mutations in arabidopsis are lethal, and their lethal action is usually timed to the sprouting phase. Moreover, in arabidopsis we know of another extensive class of dominant and recessive embryonic lethals whose action reduces to curtailment of development and death of embryos at different stages of embryogenesis. The first mutations of this type were found by Mueller (1961a); he also developed their classification by phenotype and a method for their quantitative analysis in immature pods(Mueller, 1963). The general view of pods containing embryonic lethals is shown in Fig. 5. Embryonical lethals are the most extensive class of the mutations known in arabidopsis. Thus, with the same dosages of mutagenes, they may be found several times more often than chlorophyllic mutations. It is therefore no surprise that embryonic lethals have most often been studied in works on experimental mutagenics, both radiation (Mueller, 1965b, f, h, 1966a; Ivanov et al., 1969, 1970; Ivanov, 1970; Sanina, 1970; Sanina et al., 1970; Usmanov, Mueller, 1970; Ginter, Ivanov, 1971; Timofeyev-Resovskiy et al.,

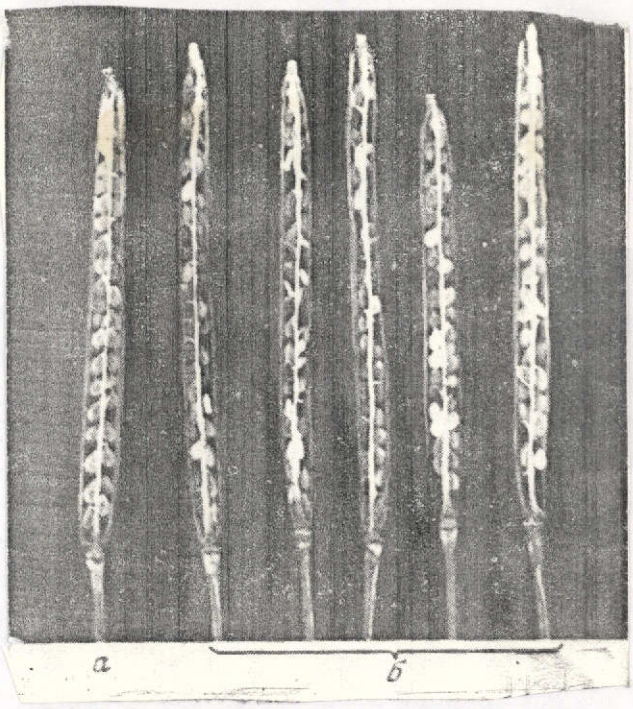



Fig. 5. Immature pods of normal arabidopsis plants (a) and heterozygotic plants in embryonic lethals (b).

1971) and chemical (Mueller, 1965a, c, d, g; 1966, 1966b, d, e, f, 1967a, b, c, 1969, 1969a, 1971; Mueller, Gichner, 1964; Van der Veen, 1968; Van der Veen, Heemert, 1968). At the same time, the genetic nature of embryonic lethals is still not precisely known, and studies devoted to research of this process appear quite rarely (Mueller, 1961; Mueller, Heydecker, 1968; Brown et al., 1965; Van der Veen, 1967, 1967a). We can only imagine that similar to the vast classes of lethal mutations in other objects, embryonic lethals in arabidopsis are a mixture of gene and chromosome mutations.

In Ch. 14, in discussing the problem of the interrelationships between genetic and somatic effects of irradiation of arabidopsis, it will be proven that the main contribution to the total mass of radiation-induced embryonic lethals is made by apparent chromosome mutations.

As in other plants, in arabidopsis, in addition to the /22
mutation types briefly examined above, mutations may occur which affect the course of meiosis and either lead to partial or complete sterility both of the male (Redei, 1964a; Dankert, Contant, 1968; Mueller, 1968; Van der Veen, Wirtz, 1968) and female (Redei, 1964, 1964b, 1965) or to disturbances of the distribution of chromosomes in meiosis (Redei, 1965; Li, 1967). All these cases of hereditary sterility in arabidopsis are related to so-called nuclear sterility and are mainly caused by recessive mutations. For some of them we know of mutations which restore the fertility of the plants (Van der Veen, de Jong, 1969). No cases of cytoplasmic male sterility in arabidopsis have been described.

Finally, aside from gamete mutations, somatic mutations can be derived in arabidopsis which are both chlorophyllic (Gichner, Veleminsky, 1965) and morphologic (Fujii, 1964 et passim); cases of somatic recombination of genetic markers have also been described (Hirono, Redei, 1963, 1965, 1965a; Hirono, 1964a, 1965).

Therefore, in arabidopsis many different mutations have been/23 found and intensely studied. And this fact, together with the aforementioned features of arabidopsis (short life cycle, large seed output, small number of chromosomes, self-pollination, simplicity of crossing, and ease of mass laboratory raising of arabidopsis) makes this plant one of the most promising botanical objects of genetic research; research already completed shows the promise of arabidopsis for research in the most varied fields of modern genetics.

Some of the mutant forms of arabidopsis can be of interest to aerospace biology.

## 2.3. Basic Trends in Genetic Research of A. thaliana

In the field of molecular genetics, research on arabidopsis has still not emerged from the preparatory methods phase. Most work in this direction is devoted to methods of isolating nucleic acids and proteins of arabidopsis and their constituents (Veleminsky et al., 1964, 1965; Jacobs, 1965c; Bhatia, 1967b; Kuo, Redei, 1971; Redei, Kuo, 1971) and also to the study of their physio-chemical and biochemical properties (Redei, 1967c; Abeels, 1968; Abeels, Digneffe, 1969; Abeels, Dubois, 1969; Abeels et al., 1970; Bonotto, Jacobs, 1968, 1969). We can think that these works serve as a basis for conducting inherently molecular-genetic study of arabidopsis in the near future. Now we can only point out a small number of studies on DNA biosynthesis in arabidopsis at different phases of ontogeny (Wibaut, 1966; Brown, 1967a; Jacobs, Bonotto, 1968; Ledoux, Jacobs, 1969) and attempts to construct molecular-genetic models of phenomena of heterosis(Langridge, 1962; Redei, 1962a; Li, Redei, 1969c).

/True, we only spoke briefly about research on phenogenetics and genetics of arabidopsis evolution, in introducing chlorophyllic mutations. Here we can only once again stress that one of the most promising trends in this field can be considered the study of the phenogenesis of photosynthesis of plants based on comparative research of chlorophyllic mutants of arabidopsis (Nasyrov, Kasyanenko, 1965; Kasyanenko, 1966, 1969; Kasyanenko, Giller, 1967; Giller et al., 1971; Kasyanenko, Nasyrov, 1968, 1968a; Kasyenenko, Usmanov, 1969; Kranz, 1968, 1970). We can also think that now when genetics of development is becoming one of the central fields not only of genetics but of biology in general, arabidopsis can become a beneficial object for studying mechanisms of genetic control of the main processes of ontogeny--determination, differentiation and morphogenesis. Research in this direction is still very limited (Westerman, Lawrence, 1970; Westerman, 1971, 1971a, b; Grover, Byrne, 1972; Jacobs, Schwind, 1972).

Population-genetic research has also been started on arabi- /24
dopsis. This direction may be one of the most promising since there is virtually no population genetics of autogamous forms yet (with the relatively high development of theoretical and experimental genetics of populations of bisexual crossed fertilizing organisms).

Naturally one of the first problems which should be solved in population genetics of arabidopsis is the question on how valid is the assumption on the obligatoriness of self-pollination in natural populations of arabidopsis. For this purpose, various authors studied variability in $F_1$ from individual collections in natural populations, using as the basic feature the length of development from rises to flowering or features associated with it. The findings accordingly indicated that natural populations contain both purely early and purely late forms and also forms splitting in this feature; the frequency of splitting families is quite high--25% or more (Napp-Zinn, 1964; Dobrovolna, 1967; Effmertova, Cetl, 1968; Jones, 1968; Karbe, Roebbelen, 1968; Karlovska, 1970; Trojan, 1971).

From these observations a conclusion was made that the high frequency of heterozygotes in natural populations is caused by the high frequency of cross pollination. But this conclusion can hardly be convincing enough, since the authors, in selecting material from natural populations, knew nothing about the time period and the processes involved in the accumulation of the large number of heterozygotes in these populations. In reality, in tests more suited for evaluating the frequency of cross pollination, which we mentioned earlier, when frequency of self-pollination was defined in a dense mixed sowing of marked lines, it was found that the frequency of self-pollination was on the order of 1% (Lawrence, Snape, 1971; Roebbelen, 1971). Therefore, the high frequency of heterozygotes in populations of arabidopsis can not be attributed to the high frequency of cross pollination, but more likely to the accumulation of these heterozygotes due to population-genetic processes. Study of the structure of populations of Arabidopsis thaliana, systems of crossing in them and the effect on the parameters of the populations of the yarovization process has just been started of late (Jones, 1971, 1971a, b; Snape, Lawrence, 1971); it has still not yielded any interesting results. We can only speak of the laying of the foundations for future study of population-genetic processes.

Analysis of variability and genotypic composition of natural populations of arabidopsis are the subjects of virtually all other works on population genetics of this plant (Seyffert, 1960; Cetl, 1965, 1969; Cetl et al., 1965, 1968, 1969; Cetl, Dobrovolna, 1968; Dobrovola, 1967, 1968; Bliss, Roebbelen, 1969; Ashraf, 1970; Fershtat, 1971). A general finding of this research--establishment of

adaptability of arabidopsis populations (in any event in periods /25
of development) to climatic and meteorologic conditions of their habitation.

As was already noted above, the main part of genetic studies on arabidopsis have been devoted to the study of mutagenesis. For illustration it suffices to indicate that the total number of publications on chemical mutagenesis is approaching a hundred; and on radiation--50; some of these studies (a small number) are devoted to comparative study of radiation and chemical mutagenesis.

Among the studies on chemical mutagenesis in arabidopsis, the leading place belongs to research of the comparative mutagenic effectiveness of diverse nitrous compounds--a vast class of mutagens, first described by I. A. Rapoport (1948). In the numerous works of different authors (especially from Czechoslovakia) dozens of nitrous amines have been tested and it has been found that almost all of them have high mutagenic effectiveness with respect to inducing embryonic lethals, chlorophyllic mutations and also mutations of quantitative features (Gichner, 1965; Gichner, Veleminsky, 1967, 1970; Kucera, 1965; 1966; Cetl et al., 1966; Boelpaepe, 1968; Veleminsky et al., 1967; Veleminsky, Gichner, 1968a and others). We know that the mutagenic effectiveness is not possessed by only nitrous compounds, but by by-products of their partial hydrolysis. This fact explains the relationship of mutagenic effectiveness of nitrous compounds as functions of their stability: the most effective are mainly unstable compounds, but not too rapidly hydrlyzing, since active by-products of the latter apparently do not reach the chromosomes of the germ cells. The presence of this "optimum instability" explains the effect on the mutagenic effectiveness of nitrous compounds of such factors as pH (Gichner, Veleminsky, 1968; Veleminsky, Gichner, 1970); metabolic inhibitors (Mueller, 1965c, Veleminsky et al., 1965), EDTA (Mueller, 1966e) and some others (Veleminsky et al., 1967, 1968; Gichner, Veleminsky, 1971). No connections of a more general nature have been detected between the molecular structure of nitrous compounds and their mutagenic effectiveness.

Data on the mutagenic effectiveness of nitrous compound in arabidopsis can be found in the reviews (Roebbelen, 1962b; Veleminsky, Gichner, 1968) and general data on them in the collection "Supermutagens" (Rapoport, Ed., 1966).

But not one of the nitrous compounds has been studied in arabidopsis as detailedly as ethylmethanesulfonate (EMS) and a small group of related substances (Gichner et al., 1968; 1968a; Jacobs, 1964a; Mueller, 1969a). It was found that EMS is so /26
highly effective in arabidopsis, as in other plants, animals and microorganisms, and can elicit in arabidopsis all known types of mutations (cf above). The high mutagenic effectiveness of EMS combines with its low toxicity, so that the maximum

possible yield of mutations is defined not by the death of M1-plants, but by induced sterility (Mueller, 1966, 1966b). In the development of plants (beginning with seed swelling), their sensitivity to EMS (with respect to induction of mutations) varies, but not smoothly; peaks of the greatest sensitivity come at the end of swelling (5-6 hours at room temperature), the beginning of the first mitoses and the period preceeding gametogenesis (Roebbelen, 1965a, 1966, 1966c; Mueller, 1967a, b). As in other chemical mutagencs, EMS efficiency depends on the conditions of its application. So, for exampl,e it increases if EMS is applied in the form of a solution in dimethyl-sulfoxide (Bhatia, 1967, 1967a) and also in the presence of EDTA (Gichner, Veleminsky, 1965a) or ions of copper or zinc (Bhatia, Narayanan, 1965, 1965a) and drops in storage of seeds dried after mutagenic treatment (Mueller, 1966, 1966b; Van der Veen, van Heemert, 1968).

The most interesting, but also infrequent works on chemical mutagenesis in arabidopsis are the studies on the mutagenic effect of analogs of nucleic acid bases. Interest in analogs of bases is defined by the fact that they can induce only genic mutations and, consequently tests using them have played no small role in development of foundations of molecular genetics. In tests on arabidopsis effects studied were mainly induced by 5-bromdesoxy-uridine, 5-iododesoxyuridine, 8-asaguanine and orange acridine. Genetic tests on arabidopsis with base analogs are made difficult by their high toxicity both in introduction to the medium and in application to the point of growth (Brown, 1962, 1967; Jacobs, 1965b, 1967, 1968; Corcos, 1970; James, 1970). At sufficiently low concentrations of analogs, they are incorporated by the plants and are included into the DNA of the cells of the apical meristem (Brown, Smith, 1964; Gavazzi, Redei, 1971). In the offspring of plants which have incorporated analogs of bases, discrete mutations may appear leading to retarded development of the plants, and isolated chlorophyllic mutations (Jacobs, 1964, 1967a, 1969a; c; Jacobs, Bonotto, 1967; Hirono, Smith, 1968, 1968a, 1969). As pertains to the frequencies of chlorophyllic mutations and embryonic lethals, then (with similar test conditions to the preceeding) under the effect of the base analogs the yield of these type mutations does not increase (Roebbelen, 1964b; Mueller, 1965g). This fact can be seen, apparently, as an indirect indication of the fact that among chlorophyllic and embryonic mutations, the portion of genic mutations is relatively small; most often, they appear as chromosomic mutations.

In addition to the aforesaid, in arabidopsis several types /27 of other work have been conducted on chemical mutagenesis. But they are all the results of fragmentary tests on the mutagenic effectiveness of different organic compounds, and for that reason they can hardly be worth discussing.

The origin of radiation genetics of arabidopsis, as we said before, was posited by the work of Reinholz (Reinholz, 1947) who produced about two dozen morphologic mutations in radiation of seeds by X-rays. About 50 studies have been done since then on radiation genetics of arabidopsis. Tests differing in various aspects of radiation-genetics on arabidopsis are discussed in chapters of the third part of this book. Here we can limit ourselves only to the enumeration of a few general findings.

In most experiments, seeds of arabidopsis were irradiated with various doses of X- or gamma-rays. As in tests with other organisms, due to irradiation all known mutation types in arabidopsis were produced: morphologic (Reinholz, 1947, 1947a; McKelvie, 1961, 1963), chlorophyllic (Roebbelen, 1957; Kasyanenko, 1965; Ivanov, 1971), biochemical (Langridge, 1955, 1958), lethal (Mueller, 1961a, 1963, 1966a; Veleminsky et al., 1964), cytoplasmic (Roebbelen, 1962, 1964; Arnold, Cruse, 1966) and also gene mutations controlling quantitative features (Daly, 1960a; Brock, 1965, 1967, 1967a), hypoploidal genomic mutations (Arnold, 1964; Arnold, Cruse, 1965) and finally, somatic morphologic (Fujii, 1964) and chlorophyllic (Gichner, Veleminsky, 1965) mutations.

With the action on seeds of arabidopsis of emissions with different linear losses of energy (LEE), the frequency of different types of mutations first increases in direct proportion to LEE (to several times versus standard rigid X-rays or gamma-radiation) [Daly, 1961; Fujii, 1964, 1966; Ivanov et al., 1967, 1970; Ivanov, 1970; Ginter, Ivanov, 1971; Timofeyev-Resovskiy et al., 1971; Hirono et al., 1967; Smith et al., 1969] and then at very great values of LEE (alpha-particles, heavy ions) drops, approaching the level of X-rays and gamma-rays (Fujii, 1966, 1969; Fujii et al., 1966, 1967; Ivanov et al., 1969; Smith et al., 1969; Hirono et al., 1970).

Of other factors capable of affecting the yield of induced mutations, in tests on arabidopsis were studied: growth of plants at moment of irradiation, post-radiation seed storage, thermal shocks, metabolic inhibitors.

In irradiation of plants at various phase of growth and development, comparison of the yield of induced mutations is complicated by the fact that as there is an increase in the plants the correlation between the frequency of appearance of induced mutations in cells of the meristem $M_1$ and the frequency of splitting of mutants /28 in the $M_2$ generation changes; the dynamics of this change, governed by the dynamics of development of the generative meristem, remains unstudied. Possible approaches to this question are discussed in Ch. III. It is clear that this methodologic problem, in tests on the yield of induced mutations at different stages of ontogeny of plants, are usually studied either at its early stage--from dormant seed to inception of cell division in germination (Roebbelen, 1960, 1960a, 1965a; Ivanov et al., 1969) or very late sporo- and gametogenesis (Roebbelen, 1965; Mueller, 1965b; Usmanov, Mueller, 1970). In swelling and sprouting of seeds of arabidopsis, the yield of

induced mutations , as usual in plants, increases and then stabilizes (Roebbelen, 1965; Ivanov et al., 1969). In comparative study of the frequency of chlorophyllic mutations induced in ovaries, pollen and zygotes, most sensitive to irradiation were the zygotes; followed by the ovaries;least sensitive was the pollen (Roebbelen, 1965) which is apparently due to the different irrigation properties and different chromosome state in the irradiated cells. The frequency of embryonic lethals and chlorophyllic mutations in arabidopsis negligibly varies in the 2-week post-radiation storage of dormant seeds (Sanina, 1970; Sanina et al., 1970) but it increases noticeably in storage for 75 hours of doubly dried seeds irradiated in swollen state (Mueller, 1967c). There is little change in the yield of induced mutations in arabidopsis and in the effect of thermal shocks before or after irradiation (Nikolov, 1968; Nikolov, Ivanov, 1969) and also in the effect on seeds during and after irradiation by metabolic inhibitor $NaN_3$ (Mueller, 1966c).

Finally, some studies on mutagenesis of arabidopsis were specially devoted to comparing the effectiveness of ionizing radiations and chemical mutagens (Roebbelen, 1962c; Mueller, 1965f, h; Jacobs, 1969b; Kawai, 1969; Redei, Li 1969a). Separate information on this can be found in many other works. In examining these studies we must remember that comparison of effectiveness of radiation and chemical mutagens acquires meaning only if different effects of the mutagenic factors employed are being compared, e.g., correlation of different types of mutations or ratio of frequency of mutations to sterility, lethal effect, etc. Then results of comparison can be interesting both from the viewpoint of mechanisms of action of the compared mutagenic factors and from the viewpoint of the nature of events induced thereby. More details will be discussed in Ch. 14.

It could have been possible to cite many more examples of the successful use of arabidopsis as an object of research in different fields of genetics and general and experimental biology, but this /29 is already enough to state that this plant has answered the hopes placed on it; it is certainly one of the most promising new multicellular objects worthy of fixed attention and thorough study.

But as already noted in the introduction, in working with new objects certain problems usually arise due to the fact that the broad front of specialized research often anticipates less effective but completely necessary study of the basic properties and features of this object. This is valid with respect to the arabidopsis. Let us illustrate this with two examples.

First example--composition of genetic charts of arabidopsis. In this plant several hundred mutations are already known which are intensively being used in special research on genetics of development, phenogenetics, biochemical and molecular genetics, population genetics, radiation and chemical genetics. But in

taking stock and genetic analysis of this vast fund only the beginning has been made and we are still quite far from the necessary orderliness which was attained long ago in genetics of drosophila (Bridges, Brehme, 1944; Lindsley, Grell, 1968) and Maize (Eyster, 1934).

An origin to the structure of genetic charts of arabidopsis was posited by the research of McKelvie (1962, 1965) who, after analyzing results of many paired crossings between derivatives of 190 morphologic mutations, established 4 groups of coupling: one large including 15 locuses with pre-evaluation of distances between them; two groups of 4 locuses each and one having only 2 locuses. Parallel to the study of groups of coupling in arabidopsis--the work by Redei et al., (Redei, Hirono, 1964), who isolated 6 short groups of coupling: 1 with four locuses; 1 with three; 3 with two and six (extra) with only one locus; in no one group did the maximum distance between locuses exceed 40 units, so that it is difficult to ascertain their independent status. With the aid of trisomes it was possible to identify the 2nd and 3rd group of coupling of McKelvie with the 3rd and 5th groups of Redei (Lee-Chen, Buerger, 1967), the other groups still remain unconnected. Therefore, work on charting genes in arabidopsis is just beginning, and this greatly complicates identification and comparison of newly obtained mutations with previously described ones; consequently, it reduces the value of subtle specialized research. Meanwhile, the tediousness, laboriousness and absence of rapid and effective results in studies on the genetic charting of arabidopsis still continue to fully divert the attention of researchers into special aspects of genetic analysis--such as analysis of the structure of discrete /30 polygenic complexs controlling the inheritance of quantitative features (Hussein, 1968, 1969, 1970; Hussein, Van der Veen, 1968), analysis of the delicate gene structure (Redei, 1964c, 1971; Li, Redei, 1969b; Buerger, Roebbelen, 1970) etc., and only quite rarely do publications appear devoted to genetic analysis of arabidopsis (Lee-Chen, Steinitz-Sears, 1964; Steinitz-Sears, Lee-Chen, 1968, 1970; Batalov, Kvitko, 1971, 1971a; Batalov et al., 1972; Kasyanenko, 1971; Roebbelen, 1972). This, of course, is very bad since the absence of genetic charts certainly lowers the value and internal agreement of studies on arabidopsis genetics. A great step forward in genetic analysis of mutations of arabidopsis could be made by universities having at their disposal a vast army of genetics students--such work would have tremendous pedagogic value for them and at the same time would keep them interested with its considerable element of novelty.

Other example of the lag in phenomenology from special research --radiation genetics of arabidopsis.

In the first 10-15 years of its development, interesting data were produced and published on the nature of radiation-induced chlorophyllic (Roebbelen, 1957) and cytoplasmic (Roebbelen, 1962)

mutations in arabidopsis, on the relative effectiveness of different radiations in inducing quantitative variation (Daly, 1960a, 1961) and somatic mutations (Fujii, 1964), on the comparative effectiveness and interaction of radiation and chemical mutagens (McKelvie, 1961, 1963; Roebbelen, 1962c), and so forth. Meanwhile, radiobiologic foundations of radiation genetics of arabidopsis were limited to a single study (Buiatti, Lorenzoni, 1963) which at that time used a too narrow range of X-ray doses for dormant arabidopsis seeds--20-80 krad. This stimulated the author and his collaborators to set about systematizing the basic phenomena of radiobiology and radiation genetics of arabidopsis. The discrete findings of this study have been published in a series of journal articles and brief notes (Ivanov, 1967, 1969, 1971; Ivanov et al., 1967, 1968, 1968a, 1969, 1969a, b, 1971; Ivanov, Sanina, 1967; Timofeyeva-Resovskaya, Timofeyev-Resovskiy, 1967; Nikolov, 1968; Nikolov, Ivanov, 1968, 1969; Haberer et al., 1968; Sanina, 1970; Sanina et al.,1970; Ginter, Ivanov, 1971; Timofeyev-Resovskiy et al., 1971).

In addition to this, the total data obtained allow for the composition of a complete and sufficiently full radiobiologic and radiation-genetic characterization of arabidopsis. This monographic characterization is certainly interesting both to plan further radiobiologic and radiation-genetic research of arabidopsis in ground-based tests and for aerospace flight. A generalization of original data on the radiobiology and radiation genetics of arabi- /31 dopsis and their comparison with the few data of other authors forms the primary content of the third part of this book.

## Chapter 3. A. Thaliana as a Promising Object of Aerospace Biology

There is no doubt that in prolonged interplanetary voyages of the not-too-distant future, one of the obligatory companions of man on board the spaceship will be green plants. And not merely a companion, but an organic component of the biologic system of crew life-support. From the photosynthetic activity of the plants will come the regeneration of the gas composition of the ship's atmosphere; they will provide fresh green vegetables and nutrition for heterotrophic organisms making up the balanced ecologic system required for long space voyages. Consequently, it is clear that in order to be ready to create such balanced and viable biologic systems for crew life-support in spacecraft, we must carry out a great deal of preparatory work both on the effects of space flight on the proposed animal and plant components of the life-support system and on a balance in the number and biomass of types of experimental biocenoses comprising them.

Manifold research is being conducted in both these directions; the findings have been quite widely published in the literature and can scarcely be examined in this monograph which is devoted to other problems. We can only note that in addition to seeds of other species of plants, the seeds of arabidopsis have already been included in USA space programs two times as biologic test objects to study the effect of space flight on living organisms. In the first experiment, in the BIOS program, arabidopsis seeds plus seeds of many other plant species were sent into orbital flight; later the development and change of the plants produced from them were studied on Earth.

The second time, arabidopsis seeds were sent to the Moon /32
and back on board Apollo 16, where they were one of four objects in the Biostack experiment prepared by the European Work Group on Space Biophysics (Reinholz, 1972b). For this experiment, a hermetic aluminum cylinder was prepared into which mono-layers of biologic material were placed, alternating with a different type of cosmic ray track sensor (nuclear emulsions, plastics). Spores of the bacterium Bacillus subtilis, eggs of the crayfish Artemia salina, bean rootlets of Vicia faba and seeds of Arabidopsis thaliana were used as the biologic materials. Goal of the experiment: to produce data on the biologic effects of certain heavy nuclei making up cosmic radiation. Small size seeds made it possible to use in one single experiment thousands of arabidopsis seeds; they were attached to polymer tape and were layered with the space particle detector plates; this permitted the establishment of a precise correspondence between the tracks of individual particles and individual seeds through whose sub-

stance these tracks pass.

The effects of individual heavy nuclei on seed were ascertained by their increment, growth and evolution of plants of the $X_1$-generation, their fertility and frequency of morphologic, physiologic and biochemical mutations in the $X_1$- and $X_2$-generations. The results of this test have not yet been published but we can imagine that they will be interesting. In particular, NASA experts feel that they are necessary to evaluate radiation danger of future space flights. NASA also hopes that this experiment will aid in explaining the point flares of light of unknown origin which have been observed by astronauts in previous flights.

In no way diminishing the value of already done space experiments on arabidopsis, we should nonetheless remember that these are only the first steps; the tasks being solved are still quite modest and the broad possibilities of arabidopsis as a test plant have far from completely been utilized. In an experiment on Apollo 16, only one (and not the primary) merit of arabidopsis was utilized--the small seed size--and the effect was studied (under flight conditions) of only one factor--heavy nuclei and only on dormant seeds. This naturall meets the tasks of a given radiobiologic experiment, but it has only an indirect relationship to the study of one of the central questions of modern aerospace biology--on the effect of prolonged sojourn in space on living organisms.

The duration of forthcoming interplanetary flights, as well as the duration of work on manned space stations, will be counted in the months or even years. During this time, the plants taking part in the life-support system will complete a full cycle (and /33
perhaps more than one) of development under space flight conditions. Meanwhile, there is still no answer to the question as to how these conditions will affect the growth, development, morphogenesis of plants, and the formation of seeds and fruits in them. And this answer can not be obtained in experiments on dormant seeds. It would also be highly ineffectual to attempt to obtain an answer on the effect of prolonged stay in space on the entire plant development cycle using large or slowly-developing forms. On the other hand, we imagine that the most suitable object for this purpose is a small, rapidly developing plant which can grow in purely synthetic media of constant composition and preferably under sterile conditions. Then the appropriate experiment will not require too much room and will not need a very long space flight. All these requirements are satisfied by Arabidopsis thaliana of all known objects of experimental botany; and especially the ephemeral forms of the species.

The effect of most space flight factors--launch loads,

vibrations, radiation--can more or less be accurately simulated under ground-based conditions and by results obtained one can give a sort of referential prediction of expected effects under actual flight conditions. In addition, the effect of this unconditional factor of prolonged space flights, the prolonged state of weightlessness, does not yield to experimental simulation in ground laboratories. Moreover, to predict prolonged space flights we must have, as we said before, data not only on the effects of weightlessness on the adult organism, but also on the processes of individual development of organisms making up the biologic environment of the astronauts. This is particularly important because if we can expect any specific effects of weightlessness on phenomena of viability, these effects must mainly be detected in biomechanical phenomena: in movements of cells and intracellular structures in processes of oriented growth and morphogenesis.

It is here that some of arabidopsis's features are most profitable, making this plant an important object of experimental genetics, which we already mentioned, and those attainments in developing methods of arabidopsis cultivation which will be discussed in forthcoming chapters.

Let us briefly discuss the merits and deficiencies of arabidopsis as an object of aerospace biologic research.

One of the main merits of arabidopsis as an object of aerospace research is the small size of most natural, rapidly developing forms and laboratory lines of this plant. The value of this feature for space experimentation goes without saying. /34 Among the natural forms of arabidopsis such ones are common whose height by the time of maturation is only several centimeters (8-10) with a rosette diameter of 1.5-2 cm. Mutant dwarfs are even more compact, with a total height of 2-3 cm and rosette diameter of 0.5-1 cm.

To plan space experiments encompassing the entire life cycle of plants, an advantageous feature of arabidopsis is the wide range of variation in duration of the vegetation period in different natural and laboratory forms of this species. This wide variation facilitates the combination of experiments on arabidopsis (by selecting the necessary forms) with experiments on other objects. Among known forms of arabidopsis there are those which differ by their virtually record rate of development among all higher plants, so that the complete life cycle may take place within the confines of a month. Moreover, possibilities of selection for the rate of development of plants of arabidopsis have yet to be touched upon.

In favor of arabidopsis as a promising object of aerospace

research is the tolerance of this plant to conditions of root nutrition and the possiblity of its cultivation in the most diverse nutritive media--liquid and solid, natural and artificial. This will certainly aid in a structural solution to experiments with vegetating plants.

As with any other typical higher plant, arabidopsis constructs its biomass during photosynthesis and consequently, needs light. Being a long day plant, arabidopsis requires a light photoperiod of at least 14 yours/day to convert to generative development.

In addition to photosynthesis, light plays a large derivational role in the life of plants: along with geotropism, positive phototropism of subterranean organs and negative phototropism of the roots have a considerable value in the spatial orientation of the growth processes. Consequently, in space experiments with vegetating plants of arabidopsis two important processes may be studied. First, in experiments with normal photoautotrophic green forms, we can study whether the phototropic orientation of the growth processes suffices to ensure the normal form of growth under weightless conditions. The solution to this problem is primarily of practical value to the astronauts. Secondly, the presence in arabidopsis of auxotrophic mutations which cannot perform photosynthesis but can develop in special organic media without light permits us to pose the question of the possbility of normal (or impossibility) course of growth processes and derivation, if we preclude both factors of spatial orientation--both phototropism and geotropism. The solution to this problem has not merely applied but also theoretical value.

In favor of arabidopsis as a promising object of space /35
research is the obligate self-pollination in this plant. In reality, space flight, primarily the state of weightlessness, should hinder the efficient pollination of flowers of higher plants. This particular concerns cross-pollinated plants which normally have entomophilic or anemophilic pollination. More chances for successful pollination and subsequent bonding of seeds and fruits in plants would be had by obligator self-pollinators. Arabidopsis is just such a plant. While self-pollination in this plant is normally done before dehiscence of the flowers (in some forms at least), the scattering of pollen in its impossible settling in weightlessness on the pistil stigma should be minimal.

Finally, of the merits of arabidopsis as an object of research of space biology we should also note the presence in this species of a wide diversity of already known mutants. As we noted in Ch. 2, the mutants known in arabidopsis encompass an extremely wide spectrum of morphologic, physiologic and bio-

chemical hereditary changes. The vast collection of mutant forms permits each time to select test objects which in the best manner meet the requirements of specific research.

Naturally, each individually-taken feature of arabidopsis discussed here as a merit of this plant as an object for research of space biology can be found in many other vegetative species from the number of higher plants. But it is hard to name any other plant from the number of sufficiently well studied objects of experimental botany which has all these favorable features combined as successfully as in arabidopsis.

We can, of course, object that arabidopsis is not promising for inclusion in the ecological life-support system of the space crew. Actually, the biomass of arabidopsis is of no food value for man. It is also valid that as an oxygen regenerator, arabidopsis is inferior to other plant species both from the order of higher plants and from algae. But one thing--utiliarian requirements, the other is scientific. Certainly with the aid of arabidopsis can efficiently and economically produce an extremely valuable and full information on conditions of viability of high plants in prolonged space flights. This information will greatly simplify both the reasonable planning of applied research (even on other objects) and the solution of purely practical problems of aerospace engineering.

In concluding this small chapter, we must note that the /36
problem of rapid and effective incorporation of arabidopsis as an object of research of aerospace biology is greatly simplified due to achievements in ground-based research of this plant. This mainly concerns the high level of development of current methods of experimental research of arabidopsis which are discussed in the second part of this book. To an even greater extent, this is related to research on radiobiology and radiation genetics of arabidopsis stated in the third part of the book. And research in this field is value not only for the actual results derived, which can serve at first glance as a basis for comparison with data obtained in space experiments. No less, of not greater value may be possessed by the very structure of radiobiologic and radiation-genetic research of arabidopsis: complex study of the effect of irradiation on survival, growth, development, fertility and mutation of arabidopsis can be used as a prototype of an optimal plan of space experiments on this plant. This is precisely why this fully "ground-based" monograph was given for publication in "Problems of aerospace biology."

## Part II Methods of Laboratory Research of A. thaliana

It goes without saying that methods of experimental work with /37 arabidopsis have much in common with similar methods employed in the study of other higher plants. At the same time, the specific nature of this new object of experimental botany and the originality of some trends of investigation started with it required the creation of a series of new (or at least a modification of previously known) methods. This concerns the cultivation of plants, the quantitative recording of their somatic and genetic features, and the staging of different types of experiments. Therefore, having in mind the promise of arabidopsis' introduction into new fields of research in particular, including aerospace biology, it seems desirable to state here the basic knowledge of methods of laboratory work with this plant. In connection with the questions investigated in this book on the radiobiology and genetics of arabidopsis, the second part of the book is divided into several chapters. In Chapter 4, the basic methods of cultivation of plants, including the different variants of aseptic culture, are examined. Chapter 5 is devoted to a description of the phenology of arabidopsis and to the methods of calculation based on somatic features (survival of plants, their growth, evolution and fertility). In Chapter 6 the general foundations of methods of radiobiologic experimentation are set forth. Finally, Chapters 7 and 8 are devoted to methods of genetic research. In addition, in Ch. 7, the methods of detection of basic types of mutants (morphologic, chlorophyllic, biochemical, lethal) are discussed, but Ch. 8 is devoted to methods of numerical calculation of mutations, including a detailed analysis of the mathematical methods intended for definition of correlations between the number of mutants discovered in $M_2$ after mutagenic cultivation of seeds and the number of mutations mainly induced in the embryonic meristem of the plants M1.

## Chapter 4 Methods of Cultivation

It is possible to conduct experiments on arabidopsis both in /38 an ordinary soil culture, and using different versions of a mass aseptic culture developed for this plant. Beside this, the growth of arabidopsis throughout the life cycle in a liquid environment is possible for special purposes. The bases of methods of cultivation of the parts and tissues of arabidopsis are also worked out.

Common for all methods of cultivation of arabidopsis plants is a light, photoperiodici and temperature regimen. A. thaliana is a plant which occurs in long-day and well-illuminated regions (Laibach, 1943); therefore during its cultivation it is necessary to provide plant illumination of 5-15 thousand lux for at least 14-16 hours/day (Ivanov, 1966). Under feeble illumination the plants dilate, become weak and are readily subject to infection, but under troo strong illumination a rapid fading and withering of the leaves takes place. During a short light-day (8-12 hours), arabidopsis plants cannot pass to the generative phase of development; but if the light is strong enough they can luxuriantly vegetate for an unlimited time. This fact is sometimes used to obtain large plants from a large vegetative mass from which, if they are switched to a long light-day, it is possible to get yields of up to 40 hhousand seeds from a single plant (Mueller, 1964a; Ivanov et al., 1966). The demands for temperature conditions are different for different races of A. thaliana (Langridge, Griffing, 1959; Langridge, 1963, 1963 a): the temperature optimum for races of different geographical origin ranges from 20°C for a race originating in countries with a temperature and cool climate (e.g., Northern Europe) to 30-35°C for races originating in countries with a dry, hot climate (e.g., the Pyrenees). For the most commonly used races, Dijon, Enkheim-1, Estland, Limburg, Landsbergh (one of which, namely En-1, was used as the object of radiobiologic experiments stated below), the temperature optimum lies within the limits of 23-25°C(Langridge, 1957; Kvitko, Mueller, 1961; Ivanov et al., 1966).

Let us now discuss the basic methods of arabidopsis cultivation.

### 4.1. Soil Culture

An arabidopsis soil culture theoretically is not different from the culture of other plants. Unless it is that due to the small size of these plants one can succeed in raising a large number of them in a small area. Arabidopsis can be ground both in /39 open soil in gardens and hot-houses in ordinary thinning boxes. The main advantage of cultivation of arabidopsis is the possible yield of many abundantly fruitful plants with a sufficiently large vegetable mass when grown in open soil or under hot-house conditions (e.g., for biochemical analyses). In a temperate zone, where

the combination of a sufficiently long day with favorable day and night temperatures of rather short duration, in open soil we can not succeed in yielding more than one or two generations in the vegetation season. In the conditions of Tadzhikistan (Dushanbe), for example, we can raise arabidopsis in open soil virtually year round, although in the hottest period of the summer (daytime temperature 40-42°C) and the darkest and coldest periods in winter (10 hour day, daytime temperature is no higher than 15-18°C), the plants are noticeably depressed.

In hot-houses, with maintenance of an optimum temperature and light regimen, it is possible to raise arabidopsis year-round, yielding 8-10 generations per year. The differences in the technique of raising arabidopsis in open soil and in hot-houses are negligible, and thus in the future both cases are examined together. Usually thinning boxes of different sizes are used to raise arabidopsis. In an area of about 0.2 sq. meters, 300-400 seeds (planting depth 2-4 mm) are sown to get 150-200 fertile plants. In soil cultivation, some of the sprouts are usually lost--mainly in the cotyledonic leaf phase (partially in the rosette phase) and primarily during watering: the tender, small shoots fall under the weight of the drops of water and often die. Nevertheless, it is necessary to see that thesoil is always sufficiently moist. Moistening of the soil from below (from pans) has an unfavorable effect on the evolution of the plants, since the rising flow of the soil solution induced by evaporation leads to excessive salinization of the upper soil layer. In any event, moistening from below must not be employed for the entire cycle of evolution of the plants, but only in the beginning, until the shoots have become strong (Lawrence, 1966).

Soil for arabidopsis is prepared light and nutritious. A mixture of sandy soil and well-rotted manure in a ratio of 2:1 produces the best results. In order to avoid the appearance of arabidopsis plants from seeds which have accidentally fallen onto the soil and also weeds and pests, it is desirable to sterilize the soil (especially the upper layer which is used for mulch).

Seeds may be sown by hand, one by one, but it is more productive to sow them in a suspension of seeds from a capillary tube (Redei, 1967b); the best effect is obtained by thinning shoots grown in advance in agar together with the agar block; it is convenient to cut it with a glass rod with an internal diameter of about 0.5 cm. This last method gives a more reliable guarantee against contamination of the culture by shoots which come from seeds that have accidentally fallen onto the soil. /40

The main shortcomings of soil cultivation of arabidopsis are: slower evolution of plants (as compared to [..] cultivation which is discussed later), difficulty in creating homogeneous and stric-

Fig. 6. Arabidopsis plants of Different Ages in Test Cultures.

/41

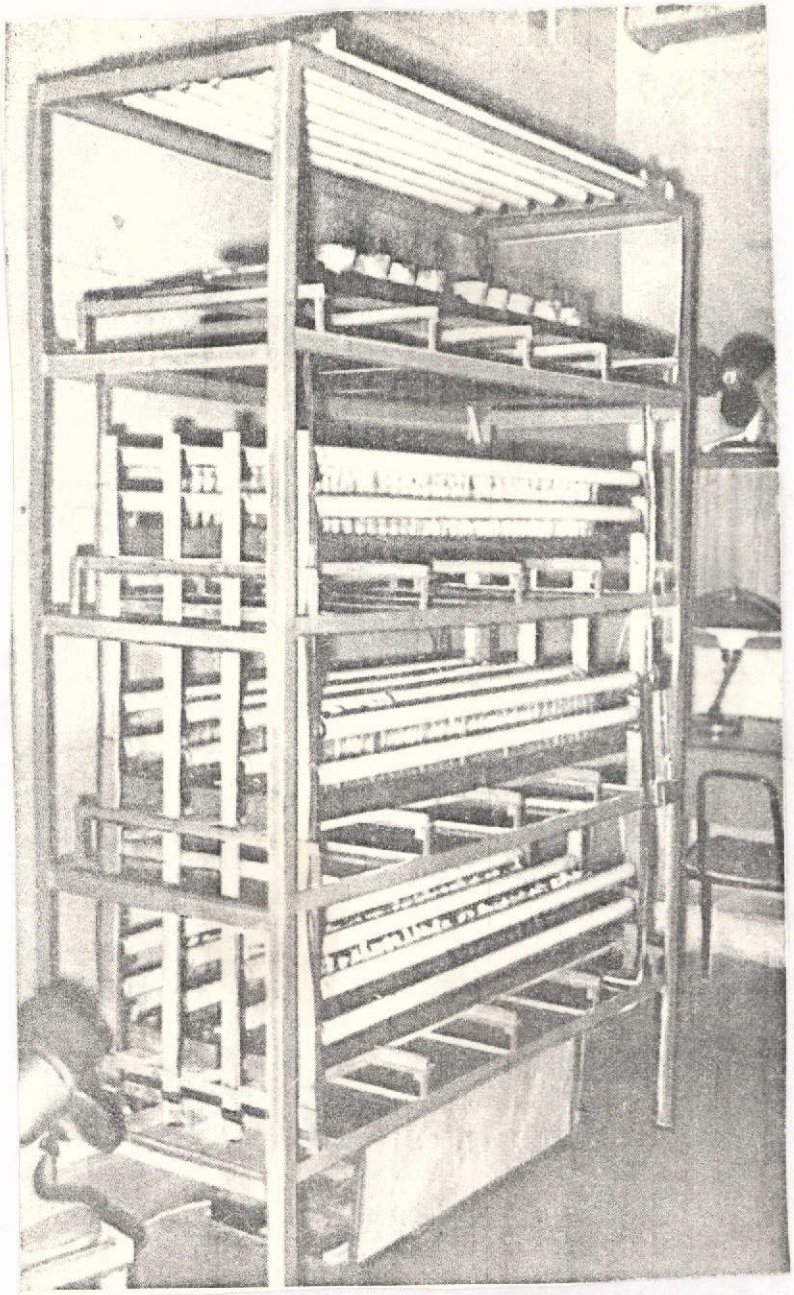


Fig. 7. Illumination Setup for Test Culture of Arabidopsis.

/42

ly controlled conditions of plant [...] (these problems may be partially overcome by using an ordinary sand culture) and extremely great variability of plan size and the rate of their growth and evolution. To obtain homogeneous soil cultures of arabidopsis, various methods have been proposed--such as the influence of cold during swelling of the seeds (Contant, 1966a; Cetl et al., 1970), planting in the soil of completely (Van der Veen, 1965b, 1967b) or partially (Contant, 1966a) etiologized sprouts, and also complex methods which include particular methods of treating vegetation vessels of special design and complex manipulation of seed and shoots (Van der Veen, 1965b; Contant, 1966, 1966a; Lawrence, 1966; Cetl et al., 1967). As a result, soil cultivation loses its primary attraction--simplicity, and the desired homogeneity does not go futher than sprouting and the early stages of plant evolution. Consequently, the main purpose of soil cultivation is to reproduce seed material;for quantitative experiments which require the maximum homogeneity of plant evolution, different versions of aseptic laboratory cultures are much more suitable.

4.2. Aseptic Cultivation

A. thaliana is easily cultivated in the laboratory with artificial illumination in ordinary thinners 150 X 15 or 160 X 16 mm in an agar mineral medium. This permits not only the achievement of the maximum standardization of conditions of cultivation, but also the maintenance of the plant for the entire evolution period under aspectic conditions. The last circumstance is especially important for isolation and study of auxotrophic mutants. Moreover, in the thinning culture the evolution of arabidopsis occurs more rapidly and the shoots virtually do not die. Thus, even when strict aseptics must be observed, the possibility of strict mass thinning of plants under homogeneous standardized conditions makes the thinning culture preferable for conducting experimental studies. Any structurally convenient version of this culture is most likely the most suitable even for conducting air-borne experiments with vegetating plants.

The methods of aseptic cultivation of individual arabidopsis plants was first suggested by F. Laibach (Laibach, 1943). Later /43
on, G. Langridge (1957) perfected Laibach's methods as applied to mass culture. All currently employed methods of aseptic cultivation of arabidopsis in thinners are essentially insignificant modifications of Langridge's methods. A general view of the plants in thinners is illustrated in Fig. 6. Cultivation of arabidopsis in thinners is best done in special illuminating units with luminescent lamps (e.g., LDTs-40 and LB-40).

A general view of such a device is shown in Fig. 7, and a diagram of its individual section in Fig. 8.

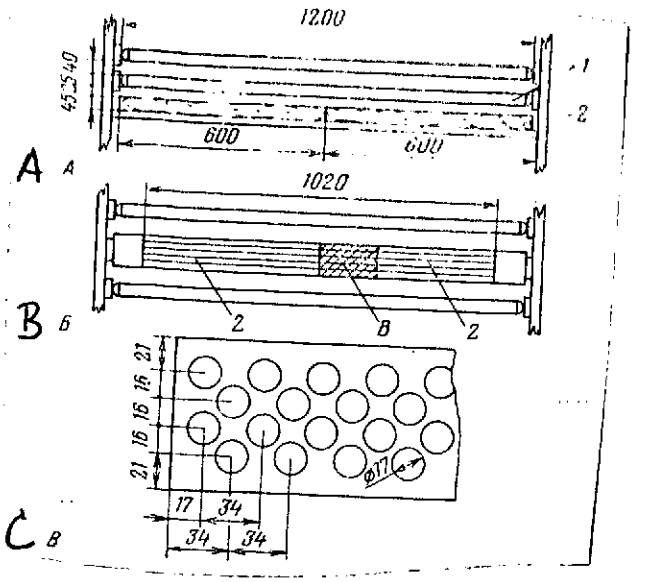



Fig. 8. Diagram of Individual Section of Illuminating Device for Thinning Cultivation. A: front view; B:top view; C: supports with sockets (top view); 1: luminescent lamps; 2) sockets; dimensions in millimeters.

In observing the dimensions indicated in Fig. 8, the necessary illumination of the plants is attained. Wooden sockets with openings under the thinner 4 cm deep are convenient for placing the thinners and plants (for thinner diameter 15-16 mm); the medium is poured flush with the surface of the socket (under such conditions the plant roots do not suffer noticeably from the light).

The nutritive mixture for the arabidopsis culture in thinners is based on a Knopp medium, enriched with microelements according to D. Arnon (1938)(Langridge, 1957) and slightly modified by K. V. Kvitko (1960) as applied to arabidopsis. The composition /44 of this medium and the order of its preparation are as follows:

Distilled water--1 liter.
Na-EDTA--1 ml 5% solution.
Macroelements, grams:

| | |
|---|---|
| $KNO_3$--1 | $Ca(NO_3)_2 \cdot 4H_2O$--0.15 |
| $MgSO_4 \cdot 7H_2O$--0.15 | $K_2HPO_4$--0.15 |
| $FeSO_4 \cdot 7H_2O$ | |

Microelements--0.5 ml solution of the following composition, %:

| | |
|---|---|
| $H_3BO_2$--0.20 | $CuSO_4 \cdot 5H_2O$--0.01 |
| $ZuSO_4$--0.025 | $(NH_4)_6 \cdot Mo_7O_{24}$--0.1 |

$MnSO_4 \cdot 7H_2O$--0.18

Agar-agar, frozen, high-grade--8-10 grams.

The amount of agar-agar depends on its batch: optimum is its [...] concentration at which the medium, cooled to room temperature, does not flow, but with energetic shaking breaks up into little clumps.

After adding each of the ingredients, the solution is carefully mixed until the sediment is dissolved. After adding the macroelements, the solution can become darker due to the formation of a thin, whitish, slightly opalescent suspension of Mg and [...] sulfates. For complete swelling of the agar-agar, the solution should be kept standing overnight (but at least 1-2 hours). Solutions of Na-EDTA and microelements can be prepared ahead of time and stored in a refrigerator. Some other recipes for arabidopsis media are known. A useful one is the medium made by I. Veleminsky and T. Gichner (1964). But, in producing identical results with the Langridge-Kvitko medium, this medium is harder to prepare.

The prepared mixture is headed to total melting of the agar and in its boiled form it poured into the thinners, which have already been set in the holders, up to the level of the socket. Afterwards, the thinners are covered with loose wads of cotton.

According to Corcos' observations (1972), the replacement of cotton wads by [..] ones noticeably accelerates the evolution of plants and maturation of seeds. Closed thinners placed directly in the racks (in order to avoid selecting the sockets of appropriate depth and diameter) are sterilized in an autoclave for 20 minutes at 1.5 atm. (after prior heating without pressure for at least 1/2 hour). After sterilization, the racks are left alone for solidification of the medium; the thinners must be in a perfectly vertical position. Observation of sterility in the thinners permits us to avoid bacterial and fungal growth which inhibit the growth and evolution of the plants and introduce another variable.

Seeds are sterilized for this same reason prior to sowing in a mixture of a 1:13% solution of hydrogen peroxide and ethanol for [...] minutes. The seeds do not have to be washed after this treatment.

Microbiologic loops are used to sow the seeds in the thinners; /45 these operate in the flame zone of an alcohol lamp and are cooled each time in a lump of prepared (sterile!) nutritive medium.

Upon completing the sowing, the racks and thinners are set in the illumination unit where they remain for the course of the experiment. The desirable conditions: photoperiods--light 18-20 hrs, darkness--4-6 hours; temperature about 25°C in light and about 20°C

in dark periods and relative humidity of 70-80%. As was established in special methods tests, these conditions are optimum for a test-tube culture of En-1 and ephemeral races similar to it. The high air humidity is required to reduce transpiration of the plants; then there is enough moisture reserve in the nutritive medium for virtually the entire cycle of development. Air humidity can be sustained in any way, including the use of simple sprayers which, when operated in combination with evaporators, also promote air cooling.

If the plants are grown in a mineral medium, the sterility can be maintained in the test-tubes only until the onset of flowering; to avoid crushing and twisting of the pods (which hinders counting of embryonic and chlorophyllic mutations) it is best to remove the specimens from the test-tubes. In this form the plants can bear fruit for quite a long time and give up to 10 or more mature pods for each 20-30 seeds, especially if little water is added to the test-tubes. After the onset of flowering, disturbance of sterility is negligible, since by that time medium infestation by microbes is no longer dangerous.

In addition to this, other versions of aseptic cultivation are known. For example, to make the medium hard, the agar can be replaced by inert loose and moisture-laden fillers such as hydro-micas (vermiculite, ceramzite, perlite; Feenstra, 1965c) or some plastics (Savin et al., 1970). It is difficult to name the advantages of such cultures, but they in turn do have an apparent shortcoming: with the regular volume of nutritive medium in the test-tube, about 5 ml excess moisture is enough for several days and must be periodically supplemented.

Sometimes the test-tubes are replaced by crystallizers, chemical dishes, Koch dishes (Ivanov et al., 1966), flasks covered with glass or polyethylene film (Velikanov, Pumpyanskaya, 1970) in which up to several dozen plants are grown together.

A special design of high (several cm) transparent covers for Petri dishes has been suggested which allows cultivation of plants until fruit bearing (Harle, 1972). The advantage of these methods is the relative ease of handling and the possibility of illuminating the plants from the side (as with test-tube cultures)/46 and from the top. But this method is less convenient when each plant has to be observed individually.

## 4.3. Special methods of cultivation

The methods of aseptic cultivation of arabidopsis described hitherto permit the cultivation of the plant for its entire life

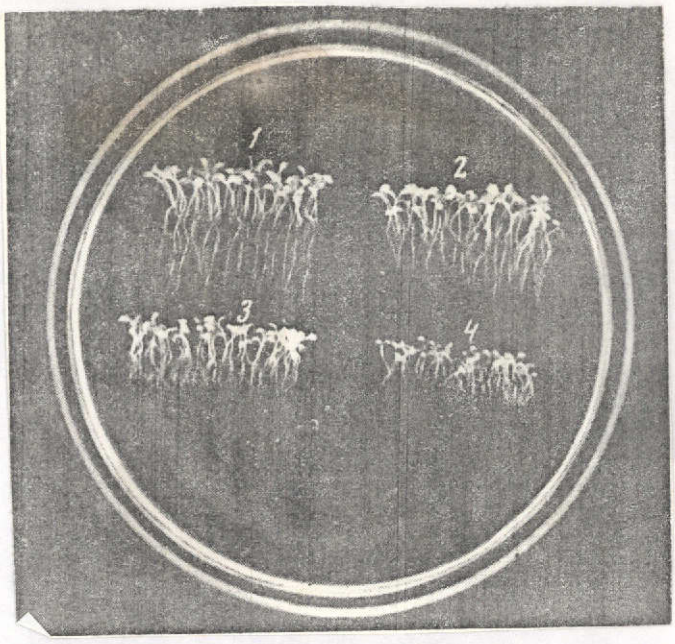



Fig. 9. Arabidopsis sprouts at Age Seven Days: 1) control group; gamma-radiation in doses: 2) 30; 3) 60; and 4) 90 krad.

cycles. But this is not always necessary. For example, a very suitable feature in arabidopsis, which permits its rapid evaluation of seed quality and their sensitivity to different chemical and physical effects is root growth. To evaluate it, it suffices to cultivate sprouts for a single week; in that time they remain so small that in a Petri dish 10 cm in diameter about 200 sprouts can be easily placed and have their roots measured. In the first week of development of the sprouts, their roots (in Di and En-1 races) reach a length of 0-20 mm; this happens to be a range where the relative truncation of the roots under the influence of various depressants (irradiation of seeds, chemical mutagens, etc.) is virtually not dependent on their absolute length in the control group (Mueller, 1964). The methods of short-term aseptic culture of arabidopsis roots in Petri dishes was first suggested by Mueller in the just mentioned work. The essence of the method is as follows. Seeds of arabidopsis (sterile!) are planted in Petri dishes in rows of 50 each, as shown in Fig.9 on several layers of filter paper moistened with a 0.1% solution of Potassium nitrate; the dishes are placed at an incline (60°) in a light box with top lighting. Sterility must be maintained to preclude fungal and bacterial infection, which sharply raises the heterogeneity of the findings. The potassium nitrate solution promotes a uniform germination of seeds. The slant of the dishes at 60° enables the optimum combination of positive geotropism and negative phototropism of the roots; in this position the roots grow along the surface of the paper, without bending away from or toward it. A week after the mass germination of seeds, measurement of sprout roots is made in each of the test versions. To do this, it is convenient to place a moist strip of millimeter paper 25-30 mm wide under several sprouts stretching each sprout with a pair of tweezers, and measuring the roots under a lens. In this procedure the measurements are more accurate since the roots are straightened out quite amply.

The moist filter paper in the dishes may be replaced by ordinary agarized nutritive medium described before; this produces greater uniformity of sprout development (our own observations

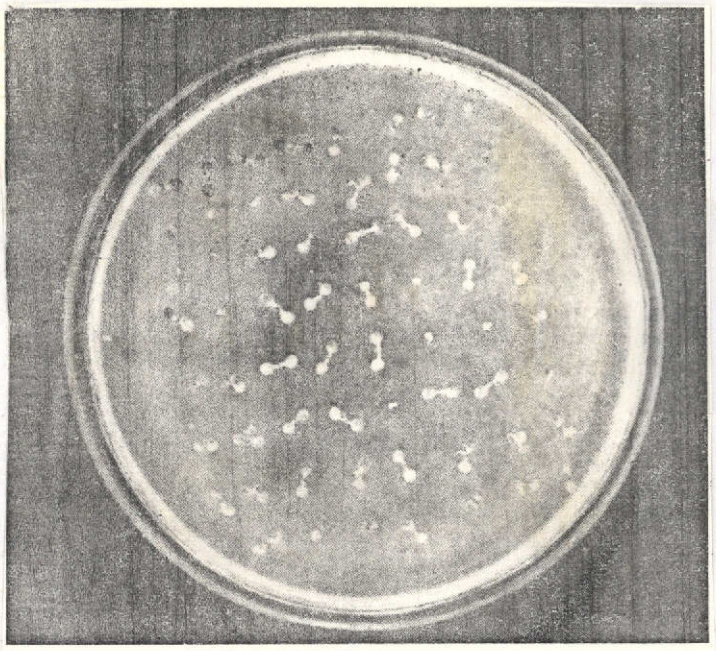


Fig. 10. Sprouts of arabidopsis from seeds splitting in mutation albina.

and also in Contant, 1966). Some authors prefer to replace the Petri dishes with chemical funnels (Bonotto, 1966) with a cut-off tube /47 covered with moist filter paper. /This funnel is set into a chemical glass of the appropriate diameter; the potassium nitrate is in contact with the filter and the top of the glass is covered by a Petri dish or a polyethylene wrap. The seeds are planted around the filter paper, leaving a 1 cm margin from the edge. Slightly better homogeneity of root growth is actually observed, but the sowing and measuring techniques are much more complicated.

In cultivating sprouts in Petri dishes according to Mueller, the dispersion of results engendered by difference between rows occupiing a different location in the dish and difffrences between dishes may be equalized by using randomized blocks to arrange the versions (Fisher, 1961, 1957; Bailey, 1962; Urbakh, 1964). It is practically adequate for each version to occupy each of the four positions on the dish but one or two times, i.e., the needed number of seeds per version is 50 X 4(8) = 200(400).

Definition of root growth can be done in a test-tube agar culture by inspecting the test-tubes and plants with illumination./48 Indeed, this can not be done when opaque fillers are used (hydromica, plastics). The inaccuracy occurring therein is due to the curvature of the roots in some sprouts--this is relatively slight. In any event, the results obtained are completely commensurate with those in Petri dishes.

In addition to analyzing growth of roots, aseptic cultures of arabidopsis in Petri dishes are also very suitable for finding chlorophyllic mutations among the sprouts which appear in the phase of cotyledon leaves. In this case the methods are very simple: etched seeds are sown uniformly in aseptic conditions on Petri dishes and are cultivated in top illumination in sealed dishes under ordinary conditions (Fig. 10).

In some instances, e.g., to manifest rare viable recombinates among a large number of lethal shoots of auxotrophic mutants, we need a rapid, dense and relatively uniform sowing in Petri dishes with a solid (agarized) medium of a large number of seeds (up to 500 per dish). This can be achieved if we first pour the seeds into a parchment disk of slightly smaller diameter than the internal diameter of the Petri dish, distribute the seeds more or less uniformly using a small brush, and then cover the disk with the seeds by an inverted Petri dish and remove the parchment using tweezers. The seeds will then adhere well to the agar and will /49 thus germinate (Mabuchi, 1972).

Of other methods of aseptic culture of whole plants of arabidopsis, we should note the so-called "swimming-pool" culture (Redei, Perry, 1971) where the entire life cycle of the plants occurs in a liquid medium (in a regular but not agarized mineral medium with 2% glucose additive). This method is interesting to study extra-root nutritiion of plants and to produce under aseptic conditions a large vegetable mass for biochemical analyses. Moreover, when the medium is enriched with the necessary metabolites, it may be useful to cultivate auxotrophic mutants.

The remarkable feature of the immersed culture is that plants of all species--Arabidopsis, Cardaminopsis and Hylandra--under such conditions change their regular photoautotrophic type of nourishment to photoorganotrophic: the growth is suppressed both in darkness and in the absence of simple carbohydrates (Redei, 1972a).

Methods of cultivation have already been developed for isolated arabidopsis embryos (Rijven, 1956), isolated roots (Neales, 1968; Weiland, Mueller, 1971, 1972) and leaf sectors (Napp-Zinn, Berset, 1966) and also methods of tissue culture (Loewenberg, 1965; Loewenberg, Thompson, 1967; Yokoyama, Jones, 1965; Ziebur, 1965; Anand, 1966; Howell, 1969; Corcos, Lewis, 1971, 1972). These methods are primarily important to study arabidopsis ontogeny or to regenerate whole fruit-bearing plants from mutant sectors. The latter is essential to study the nature of somatic recombinations.

These methods are theoretically no different from analogous

methods suggested for other plants. Study is now being done on food requirements of arabidopsis and detection of the optimum temperature and light conditions required for cultures of organs and tissues in vitro (Corcos, Lewis, 1972; Weiland, Mueller, 1972). We should note that tissue culture of arabidopsis have already permitted the reproduction of several mutant arabidopsis plants and production thereof of fruit-bearing plants (Corcos, Lewis, 1972).

Finally, for rapid reproduction of rare mutants, the method proposed by Reinholz (1972) is of interest. If during the formation of the floscule the plant is changed to the short-day, the laying of flowers in clusters is transformed into leaf rosettes, one to each flower, which can then be implanted and produce fruit bearing plants under long-day conditions.

The diversity of already available methods of arabidopsis cultivation in vivo and their parts in vitro once again stresses the promise of this new object of research in the most diverse fields of biology.

## Chapter 5 Phenologic Observations and Somatic Features

The basis for evaluating survival, growth, development and fertility of plants, herein arbitrarily united as "somatic features; and also to evaluate changes of these basic biologic parameters of state of the object under different effects (including irradiation) is constituted by phenologic observations and several measurements (root, stalk) and calculations (number of plants surviving and perishing, number of stalks, leaves, floscules, fruits, ovaries, seeds, rootlets, etc.). /50

### 5.1. Phenologic observations

Let us first discuss the phenology of arabidopsis and illustrate it using the En-1 race of A. thaliana which was used as the material of the radiobiologic and radiation genetic tests below. Let us remember that in phenologic respects this race is difficult to distinguish from other ephemeral races (Est, Di, La) used in the practice of different laboratories (Roebbelen, 1965e).

En-1 seeds used as the basis of our test seed fund had traveled a long and complex path, undergoing a series of reproductions at each stage. The natural seed material was obtained by F. Laibach from the borough of Enckheim near Frankfurt-am-Main. After many years of laboratory development the seed were given to Roebbelen at Goettingen, wherefrom they got to the department of genetics and selection of Leningrad University (K. V. Kvitko) and then to the Institute of Physiology and Biophysics of the Academy of Sciences of the Tadzhik SSR in Dushanbe(A. G. Kasyanenko); by 1965 they had reached our laboratory at the Institute of Medical Radiology in Obninsk. This "trip" took 25 years and no less number of reproductions. Thus at the present time En-1 should be considered more of a laboratory race than a natural one.

Starting in 1965, this seed fund was reproduced twice per year; for further reproduction the seeds were collected from those plants most typical of En-1 race and they were not mixed. Thus (especially when we consider that A. thaliana is a self-pollinator) the test material is made of virtually pure lines.

A general appearance of fruit-bearing A. thaliana plants is given in Fig. 2 and discrete phases of development of En-1 race plants can be seen in Figs. 6 and 11.

The following phases are clearly distinguishable in the development of arabidopsis: sprouting, cotyledon phase, rosette, budding (with stalk formation), flowering, fruit-bearing and maturation. The duration of the phase of development may vary /51


Fig. 11. Phases of Development of Race En-1. 1) cotyledon; 2)rosette; 3) budding; 4)flowering.

within very wide limits according to the intensity of illumination, the relationship of photoperiod, temperature, nutrition conditions etc. An exemplary duration of the phases of development in a test-tube culture (Ch. 4) under conditions optimal for En-1 race (temperature circa 25°C, photoperiods: light--20 hrs, dark--4 hrs; illumination 5-10 thousand lx) is cited in Table 1.

TABLE 1. LENGTH OF DEVELOPMENT PHASES OF RACE EN-1 IN TEST TUBE CULTIVATION

| Phase of development | Number of days after sowing | Phase of development | Number of days after sowing |
|---|---|---|---|
| Sprouting | 2-4 | Flowering | 18-25 |
| Rosette | 3-12 | Fruit-bearing | 21-30 |
| Budding | 13-20 | Maturation | 28-45 |

Thus, plants of the En-1 A. thaliana race are very suitable /52 for laboratory experiments: they are rather small (in an illumination area of about 0.4 square meters we can readily place 360 plants), they have a short life cycle (practically one month and an embryonic diapause no greater than two weeks--this permits production of 8-10 generations per year) and they have a high rate of seed production (under lab conditions 150-200 seeds from

one plant). This may all be used as a basis for selecting the En-1 race of A. thaliana as a suitable object for experiments.

## 5.2. Somatic Features

These features should be discussed in the sequence in which they are recorded in the course of the plants' life cycle; they are connected into feature groups of survival, growth, development and fertility of the plants. All features are discussed from the standpoint of their value in radiobiologic experiments.

### Plant Survival

We know that the death of plants due to seed irradiation can be timed to different phases of their development. It is impossible to state a priori with total determinacy how similar or different, independent or correlated are the causes of death of plants in different phases. Thus survival of plants should be recorded separately in all basic phases of their development: sprouting--cotyledon phase--rosette--budding--flowering--fruit bearing. Survival of plants in each phase of development is conveniently expressed as a percentage of the plants attaining the next phase of development out of the total number of plants entering the particular phase.

### Plant Growth

In the early radiobiologic experiments with plants it was found that plants cultivated from irradiated seeds lag in growth; this lag, which increases with the dose of radiation, concerns both the underground part and the root system of the plants.

In massive tests, plant growth of arabidopsis is conveniently described by such readily measurable features as root length and plant height at specific days of development.

In cultivating arabidopsis in a test-tube culture in agar media, the root length can be measured directly in the test-tubes by inspection against back illumination. The best time to measure root length under the aforementioned conditions is the 7th /53 day of plant development, counting from the sowing or (to reduce variability due to not fully synchronized seed germination) from their upturning. By that time the roots of the control sprouts have almost reached the bottom of the test tube (but still have not bent) and greatly differ in length from the versions irradiated with large doses; and, what is extremely important, the length of roots in the control group at this time are in a range (10-20 mm) where the effect of irradiation

expressed in percentages to the control group, is least dependent on the absolute values of root length (Mueller, 1964).

The height of plants in test-tubes can also be measured on a specific day of their development, e.g., the 21st, when virtually all control plants are in the phases of flowering and fruitbearing and have still not reached the plugs. The shortcoming of this feature as a parameter of plant growth is that in radiobiologic experiments with several doses of irradiation, not only the growth of the stems but also the growth of the plants lags differently according to the dose; thus it is impossible to seelct any fixed experiment day when the plants of all versions are in the same phase of development. Thus the height of plants measured this way reflects not only their growth but also their development. Consequently, a more precise representation on the growth of the plants is given by their height, measured in the same phase of development--most conveniently on the day that the first flower in the main floscule is blooming.

Naturally, to obtain more precise representations on the growth of the root and the underground portion of plants we must trace these processes in dynamics, defining not only growth but the rate of growth of the appropriate organs. But this precision is mostly needed only in special tests, and repeated measurements greatly increase the laboriousness of the tests. As will be seen hence, single measurements of root growth and plant height provide a completely clear picture of the relationship of plant growth as a function of dose and conditions of irradiation.

## Plant Development

Irradiation of seeds of higher plants causes two primary types of developmental disturbances. This is first a lag in passage through successive developmental phases, i.e., reduction in the rate of development and secondly, the appearance of different anomalis of development (morphoses).

In arabidopsis the most frequently encountered morphoses are multiple-stem forms and fasciations (Ivanov, 1967, 1969; Reinholz, 1972). But the appearance of both these anomalies of development is very variable and depends greatly on the fluctuation of the uncontrolled test conditions.

Rough ideas on the rate of vegetative and generative development of the plants may be obtained from phenogenetic observations on the basis of calculating such features as portion of rosettes (and consequently, portion of floscules) formed by a certain time. The rate of development of the plants can /55

more accurately be evaluated in terms of the duration of transition of successive developmental phases: sprouting--cotyledon phase--rosette--budding--flowering--fruit-bearing.

TABLE 2. BASIC SOMATIC FEATURES OF ARABIDOPSIS /54

| FEATURE | AVERAGE VALUE OF CONTROL | EXTREME VALUES IN INDIVIDUAL TEST SERIES | |
|---|---|---|---|
| SURVIVAL | | | |
| Ascent of seeds, % | 97.3 | 91.0 | 100 |
| Survival of plants(%) in phase: | | | |
| cotyledon | 93.6 | 84.8 | 98.2 |
| rosette | 96.9 | 93.2 | 98.7 |
| budding | 99.8 | 99.4 | 100 |
| flowering | 100 | 100 | 100 |
| Total survival rate of plants, % | 91.1 | 83.6 | 96.5 |
| GROWTH | | | |
| Root length, mm | | | |
| on 7th day | 11.1 | 8.6 | 13.6 |
| on 7th day after upturning of seeds | 18.6 | 12.4 | 23.9 |
| Plant height, mm | | | |
| on 21st day | 74.4 | 69.7 | 79.0 |
| on day of flowering onset | 70.4 | 56.1 | 78.0 |
| DEVELOPMENT | | | |
| Length of phases: | | | |
| sowing-ascent, days | 1.8 | 1.6 | 2.2 |
| ascent-rosette: | | | |
| % rosettes formed on 7th day of test | 73.5 | 43.5 | 92.0 |
| number of days | 10.4 | 86. | 12.1 |
| Budding-flowering,days | 7.2 | 6.4 | 7.9 |
| Flowering-maturation,dd. | 13.6 | 12.7 | 14.1 |
| Total length of vegetation period, days | 37.4 | 33.0 | 41.6 |
| FERTILITY | | | |
| Average # of seeds | | | |
| on plant | 128 | 70 | 173 |
| on pod | 24.0 | 16.1 | 31.2 |
| Sterility, % | | | |
| % sterile plants | 1.4 | 0 | 3.5 |
| % sterile pods | 30.4 | 26.0 | 36.0 |
| % of infertile seedbuds | 10.3 | 7.5 | 13.2 |

## Plant Fertility

We have known long and well that plant fertility depends on the dose and conditions of seed irradiation.

A direct estimate of the fertility of plants is the number of seeds formed. It was found that the total number of seeds in plants varies greatly in different tests, while the average number of seeds in the pod varies much less; at the same time both these features similarly depend on the dose of irrddiation. A reduction in the number of seeds can be caused both by a reduction in the number of seed layings and sterility induced by irradiation of lain seedbuds. Induced sterility can be evaluated in different ways, e.g.: as a portion of sterile plants out of those surviving, the portion of sterile pods in surviving plants, or the portion of sterile (or infertile) seedbeds of all seed layings. Each of these estimates has its own nuances, reflecting the formation of different degrees of sterility in irradiation.

Summing up the results to the discussion of calculating different somatic features of arabidopsis, we cite the values in the control group and the maximum variation of the averages in individual test series for all our test series (Table 2).

Table 2 permits the following general conclusion to be made: seeds of race En-Arabidopsis thaliana has high ascendability, and cultivation under test-tube culture conditions provides a high rate of survival, energetic growth and development and high plant fertility with moderate variation of all these features. Therefore, both the object of study and the conditions of its cultivation fully meet the general requirements of radiobiologic experimentation.

## Chapter 6. General Questions of Radiobiologic Test Methods

### 6.1. General remarks

Two circumstances are responsible for the brevity of this chapter. In the first place, the methods of radiobiologic experiments on arabidopsis do not differ in principle from the well-elaborated and well-known methods of similar experiments on other plants; and in the second place, the statement in one place of the particular details of methods different in content would detach them from a description of the obtained results. In this chapter, we will limit ourselves to a group of general problems, postponing the discussion of details and specifics to the next chapters. /56

Not infrequently we encounter projects where in radiobiologic experiments on the modifying effect of the factor in question (or factors) is judged on the basis of data obtained as a result of a single dose of radiation. But that approach can only be justified in conducting preliminary experiments or in verifying already known phenomena in specific instances; but even in those cases, the procedures are fraught with the peril of arriving at incorrect conclusions (Timofeyev-Resovskiy et al., 1968). This peril stems from the fact that the influence of factors and conditions of the experiment under study itself quite often depends on the dose of radiation. Taking this factor into account, it is necessary to set the goal in radio-biologic experiments of investigating the dose and effect curves in the maximally possible range of seed irradiation doses for each of the features.

### 6.2. Irradiation and Dosimetry

The small size of arabidopsis plants and the possibility of growing them in a 'portable' form, i.e., in test-tubes, permit the conduct of their irradiation in any phase of their life-cycle (Röbbelen, 1960, 1962a, 1965) using the customary laboratory sources of radiation. And in cases of irradiation by sources of potently penetrating radiation (strong X-rays and gamma-rays, fast neutrons), the question of the difference between surface and deep doses does not even arise.

The procedures are even better with the seeds of arabidopsis. They are so small (0.3 X 0.4 X 0.5 mm) that they are accessible to uniform irradiation by sources of radiation even of low penetrating power, such as fast protons and alpha-particles (Fujii, 1966, 1969; Fujii et al., 1966, 1966; Ivanov et al., 1968, 1968a, 1969) and even by ions of the heavier atoms, right up to argon. Another point-- /57 the seeds have to be arranged in one layer; when heavy ion radiation is used, the sprouts also must be oriented towards the flow of particles.

Finally, the arabidopsis pollen has a diameter of barely 20 microns; consequently, it is easily susceptible to irradiation by particles of even lower penetration potency. For the purpose of irradiation of pollen in a single layer, it is convenient to use velvet linings to which the pollen easily attaches and from which it can easily be transferred to the pistil stigma for artificial pollination.

As to the dosimetry of ionizing radiations, the requirements presented to it in experiments on irradiating arabidopsis do not differ from experiments on any other biologic object. Thus, in our experiments dealing with X-ray and gamma irradiation of the seeds, we utilized the customary ionizing dosimetry. In experiments with fast neutrons, the absorbed dose was calculated on the basis of measurements of the neutron flux and its spectrum in the energy range (Haberer, Regel, 1969) as well as of analyses of the element composition of the seeds. An analysis of the seeds of arabidopsis by breaking them down into their basic components was kindly performed for us in the Department of Analytic Chemistry of the Dresden Technical University; the results obtained (C = 51.2%, H = 7.8%; N = 10.5%) are in quite satisfactory agreement with the data obtained quite independently by T. Fujii (Fujii, 1964; C = = 51.7%, H = 8.2%, N = 5.2%).

## 6.3. Planning Experiments and Processing Results

We had noted earlier that the soil culture of arabidopsis is typified by very great variation of characteristics of growth and evolution of the plants which is difficult to surmount; and that the test-tube culture is much better in this respect. But even in the case of test-tube culture and in the course of working with virtually pure linear materials, the variation of characteristics still remains quite large. That is why statistical planning of experiments adequate to the changing nature of materials and the tasks of the project acquires a particularly great importance in radiobiologic experiments on arabidopsis.

Since, even within the relatively constant framework of the test-tube culture, it does not always prove possible to avoid small gradients of temperature, humidity and illumination within the test area, it follows that in setting radiobiologic experiments in such cultures we should consider the most expedient course of action to be their planning based on randomized blocks (Fisher, 1951). To set the block boundaries, it would be best to plant in the test-tubes over the entire experimental area maximally homogeneous control seeds; after analyzing the variation of properties of growth and evolution in specific sectors of the entire area (for example: outside and inside rows in the racks, the edges and middle area of the experiment room, etc.), to bring to light those sectors(blocks) whose variability is less than between the specific sectors (Ivanov /58

et al., 1966; Ivanov, Timofeyeva-Resovskaya, 1966). The best characteristics of the growth and evolution of arabidopsis that react with the highest sensitivity to fluctuations in the state of the medium are the growth of the root and the duration of the plant's evolution until the start of fluorescence (Chapter 5). It is natural that if the illumination unit is rebuilt, the block boundaries must be revised.

The problem of variation is not limited by variability within the experiments. No lesser (and, perhaps, even greater) a role is played by reproduction of the results in a series of reiterated experiments. The very first experiments dealing with gamma-radiation of arabidopsis seeds (Ivanov et al., 1967, 1968; Ivanov, Sanina, 1967; Timofeyeva-Resovskaya, Timofeyev-Resovskiy, 1967) had shown (Table 3) that the variation between repeated experiments for all the properties of survival, evolution, growth and fertility of plants is greater than within them. This circumstance pointed to the need for a series of several (relatively small in volume) repeated experiments featuring all the variants in each of them, and of accepting for the unit of observation the average values of properties obtained from individual reiterated tests, since even in cases of excessive variation of the properties we can anticipate that the distribution of averages would not contradict the normal law.

TABLE 3. RESULTS OF DISPERSION ANALYSIS OF DATA ON THE VARIATION OF SEVERAL FEATURES OF ARABIDOPSIS BETWEEN REITERATED TESTS ON GAMMA-IRRADIATION OF QUIESCENT SEEDS

| FEATURE TO BE ANALYZED | DIFFERENCES BETWEEN TESTS, P |
|---|---|
| Intergrowth | <0.001 |
| Survival of plants in phase: | |
| cotyledon | <0.001 |
| rosette | 0.05-0.1 |
| budding | 0.7-0.8 |
| florescence | <0.025 |
| Length of main root on 7th day of test | <0.005 |
| Height of plants on day 21 of test | <0.005 |
| Portion of buds by day 21 of test | <0.001 |
| Portion of rosettes by day 7 of test | <0.001 |
| Duration of vegetation period | <0.025 |
| Portion of fertile plants of survivors | <0.2-0.3 |
| Number of seeds per plant | <0.005 |

Higher variability between them than that prevailing among the experimental groups does not preclude their application in random blocks, since that scheme (in contrast to full randomization) lowers the probability of casual local clusterings of plants of a

single variant and thereby increases the reliability of the com- /59
parisons being made. As to the non-randomized placing of variants in the absence of complete homogeneity of conditions, then, as justly commented on by R. A. Fisher, this fact does not belong to the competence of statistics and a statistical processing of the results obtained in such experiments does not have sufficient grounds (Fisher, 1951). The structure of experiments which has just been described presupposes the application of dispersion analysis as the basic method of statistical processing of results. Depending on the nature of the experiments, these may be the customary plans of single or multiple factor analysis with iterations (Fisher, 1958; Bailey, 1962; Urbach, 1964, or other handbooks on statistics). In the process of analysis of qualitative properties, we should apply the angular transformation of R. A. Fisher $\phi = 2 \text{ arc sin } \sqrt{p}$, where p is the value of the feature in specific repeated experiments, %.

Finally, one more remark is in order with regard to the processing of results of our radiobiologic experiments. In the numerous dose-effect curves cited in the third section, all the characteristics for the convenience of comparison are given in percentage points of the control group. Calculation of the limits of error in all these cases was carried out on the basis of estimates of standard deviation of complex averages (Urbach, 1964, p. 119).

## Chapter 7. Methods of Genetic Research. Crossbreeding and Discovery of the Main Mutation Types.

Methods of modern genetics are numerous and varied. However, a typical genetic experiment is made up of three necessary stages: crossbreeding, discovery of mutant phenotypes and quantitative analysis. The chapters that follow deal with these problems as they apply to arabidopsis.

### 7.1. Methods of Crossbreeding

It was shown in Chapter 2 that self-pollination is the norm of sexual reproduction of arabidopsis. However, good binding of the seeds may be effected even by artifical pollination. During the crossbreedings we must observe the time of maturation of the stigma and pollen. According to the observations of A. I. Mueller (1961), the maturation of the stigma takes place even before the full blooming of the flower, roughly by the time the process reaches the edges of the still-closed sepals and petals. The receptivity of the stigma continues not just until the full blooming of the flower, but for about two days thereafter. The maturation of pollen and the opening of the anther occur somewhat later; they are typified externally by an alteration of the coloring of the anthers from green through yellow-green to yellow. The opened anthers have an orange coloration. Thus castration of the blossoms must be done before the anthers turn yellow. However, too early a castration is not only difficult to accomplish from a technical standpoint (due to the small size of the reproductive organs), but leads more often to traumatization. The small size of the blossoms requires the use of special devices--a binocular magnifying glass with a movable base, and immobilizing tweezers with soft tenons made of sponge covered with a sticky lubricant. Instead of removing the anthers by means of tweezers, the latter can be sucked out by a capillary tube connected to a water-jet pump (Feenstra, 1965d). This operation can also be done under the binocular magnifier. In that event, the diameter of the capillary tube must be adequate to suck in the anther, but must be as small as possible to limit the traumatization of the surrounding tissues of the blossom. The capillary may be replaced by an appropriate needle with a cut-off end for injections. A foot-operated control is most convenient for turning the pump on and off during the blossom castration.

/60

Artificial pollination is best administered right after the castration and, at least, no later than within the next 3-4 days, as a later date may find the stigmas beginning to wither. The rate of evolution of consecutive blossoms in the floscule permits simultaneous castration and pollination of the first three blossoms and then similar treatments for two developing blossoms on a daily basis (Mueller, 1961).

Due to the small size of the blossom, the crossbreeding of arabidopsis plants is quite a painstaking exercise and is virtually impracticable in test-tube cultivation. For this reason, it is possible (especially when a recessive form is used as the mother plant, and a dominant form is used as the father plant) to apply pollination without castration, using the heterostyle of the arabidopsis. Such pollination, done as soon as the stigma emerges from the bud, gives us hybrid seeds as a rule. Pollination is convenient to do using a whole mature anther. The observations of P. D. Usmanov have shown that the pollen of arabidopsis germinates very rapidly and as early as 5 minutes after the act of pollination (or sowing of pollen), pollen tubes reach considerable dimensions. Consequently, in the event of timely pollination (without castration) and the transfer to the stigma of an ample quantity of pollen, the possibility of subsequent pollination by other pollen (whether foreign or inherent) is virtually precluded. This fact is also confirmed by analyzing the results of crossings without castration done by A. G. Kasyanenko: the ratio of classes in the foliating offspring of such crossings doe not deviate from the anticipated norm. If we also consider the fact that the procedure of blossom castration often leads to /61 injury of the pistil tissues, it follows that the simple method of artificial pollination without castration should be considered superior (particularly if the paternal plant has any dominant characteristics) to pollination with castration. Mature pollen of the arabidopsis retains its viability for at least two months if kept under refrigeration.

It was recently discovered (Barabas, Redei, 1971) that the mutation *as* (asymmetric rosette leaves) belonging to the second group of the J. P. Redei chain (Redei, Hirono, 1964) of classification and the allelic mutation *magnifica* of A. Reinholz (Reinholz, 1947a) has a strongly expressed heterostyle: the stigmas of the pistils of the blossoms of these mutants which are susceptible to pollination jut out from the still-closed buds several days prior to the maturation of the anthers. This fact creates still better prerequisites for artificial pollination without castration when the mutation *as* is used as a maternal form; the recessive nature of leaf asymmetry, its complete penetrating nature, the constant expressiveness and sharp deviation from the norm permit an easy safeguarding of the $F_1$ offspring against self-pollination. Of course, even when working with the mutation *as*, artificial pollination should be done in time. The heterostyle of mutation *as* is even more convenient for the purpose of artificial pollination without castration of the blossoms than the nuclear or cytoplasmic male sterility (by the way, the latter situation for arabidopsis is as yet unknown), as this mutation is normally fertile and, consequently, work on it does not require the use of fertility regenerators.

## 7.2. Methods of Discovery of Basic Mutation Types

It is well known that methods of discovering mutant phenotypes are fully and entirely determined by which characteristics or properties of the organism are governed by the mutation in question. In Ch. 2, the mutations of arabidopsis were subdivided into morphologic chlorophyllic, biochemical and lethal and cytoplasmic types. Let us retain this classification here, but just combining several of the categories. The arbitrary nature of this classification belongs, first of all, to mutations having a pleiotropic effect. For example, arabidopsis is well known for its viable chlorophyllic mutations which also affect the morphologic characteristics of the plants (Nikolov, 1968; Nikolov, Ivanov, 1968, 1969) or chlorophyllic mutations lacking in synthesis of thiazole (Jacobs, 1969). But in the case of pleiotropic mutations, one may assume one of the phenotypic manifestations of the mutations as the basic one, and this will define the methods of its discovery. Consequently, the classification accepted by us will not interfer in the study of methods of discovering mutant phenotypes for arabidopsis. This classification does not comprise chromosomic and genomic mutations found by cytologic methods, but in this respect those methods used in experiments on arabidopsis are the same as those used on other plants.

### Morphologic, Chlorophyllic and Cytoplasmic Mutations /62

The methods for discovering morphologic mutations are the simplest of all--these are the customary visual observations with the naked eye or using a binocular of low magnification.

The methods of detecting chlorophyllic mutations are essentially the same. The only possible problem stems from comparison of sprouts or plants anomalous in their pigmentation with the standard color scale in order to classify them (Lamprecht, 1960, 1965). As concerns the analysis of their pigmentary composition or the study of the delicate structure of the chloroplasts, these are relevant to subsequent study of chlorophyllic mutations and not to their methods of initial detection. From the large number of diverse chlorophyllic mutations occurring in arabidopsis, the most common are the mutations *albina* (white), *xantha* (yellow), *chlorina* (yellow-green) and *viridis* (pale-green); their respective symbols are *alb*, *xa*, *ch* and *vi*. All of these are mutations which appear in the sprouts in the cotyledon phase and some of them (all *alb* and *xa* varieties, as well as some *ch* ones) are distinguishable under the magnifier even in unripened pods of maternal plants prepared from leaves 10-14 days after blooming; then, the embryogenesis is approaching consummation, but the coats of the ripening seeds are not yet pigmented and not impregnated with suberin, but are colorless and transparent (Mueller, 1963). Moreover, for methods of detection of chlorophyllic mutations, it is essential that all

*alb* and *xa*, most *ch* and some *vi* mutations be lethal in the cotyledon phase (mutations *ch* and *vi* are known which are lethal in much later stages of development).

In conformity with the peculiarities of the chlorophyllic mutations of arabidopsis which have been considered, two basic methods of detection may be employed: in the first place, in growing the sprouts (using an aseptic culture in Petri dishes in the standard agar-agar mineral medium, cf. Ch. 4 and Fig. 10), under the standard conditions of cultivation described above, the chlorophyllic mutations are then clearly visible among the sprouts on the 5th through 7th days of the experiment (Ivanov et al., 1966); in the second method, by inspection through the magnifier of unmatured pods on maternal plants, as this is done in detecting embryonic lethals (Mueller, 1963). The raising of sprouts permits the detection of many chlorophyllic mutations; but the process also involves an admixture of modified anomalies. This method makes it easy to transplant the required sprouts into test-tubes or soil to grow them until fruit-bearing for breeding or for genetic analysis in subsequent generations. In order to do this, we only have to take the sprout and a block of agar medium using a glass tube, bring it in contact with a new medium, and blow both of them onto the new medium. Such sprouts readily take root and later prove to be normally fruit-bearing.(provided, of course, that they are viable and fertile). It is true that even in inspecting unripened pods coming from green seeds we can raise fruit-bearing plants (Balkema, 1968). To do this, we should place the prepared pod, whose embryonal and chlorophyllic mutations had already been noted, in agar of usual consistency which has no traces of $KNO_3$, and cultivate it in light at an optimum temperature for arabidopsis. After 11-12 days, the seeds will sprout (their germinating ability is almost completely retained) and we can again verify the presence of chlorophyllic mutations among the sprouts. When the sprouts are stronger, we can plant them in a permanent medium and raise them until the time of fruiting. The Balkema method thus combines the resources of both methods. Moreover, it permits the elimination of the primary shortcomings of the Mueller embryonic test in its original form: the loss of seeds for propagation and further study and the lack of precision in distinguishing between chlorophyllic mutations and embryonic lethals with very late embryonal mortality (the former, by definition, should provide geminating seeds, while the latter do not); moreover, the later embryonic lethals may also have an anomalous pigmentation, so that unripened pods can be also taken for chlorophyllic mutations. It is true that among the authentic chlorophyllic mutations which basically perish in the cotyledon phase there appear to be some species where the active time of the lethal effect varies among individuals and may extend into later embryogenesis; this will be reflected in the loss of germinating qualities by the seeds in the absence of apparent external differences from normal seeds. This occurs because, in

/63

a family-by-family comparison of the germinating ability of seeds and the frequency of mutant sprouts in families splitting in terms of chlorophyllic mutations, we find a significant positive correlation between these two quantities: $r^{s} = 0.43$; $0.01 < P < 0.05$ (Ivanov, 1971). We find that from the methods discussed, the Balkema method is most labor-consuming; next comes the method of cultivating sprouts; the least labor-consuming method of all is the A. I. Mueller method. Thus, each of these methods has its advantages and deficiencies and the choice from among them depends on the purposes of the study.

And a final note: in calculating chlorophyllic mutations among the sprouts we should bear in mind the fact that the mutant seeds lose their germinating ability earlier than normal seeds (Röbbelen, 1966d) and this fact becomes apparent already after a year of storage. With much older collections (3-5 years) which had initially seen a splitting of many mutants, they may disappear altogether; and their general germinating quality is reduced in the process.

Finally, a few words about cytoplasmic mutations. All cytoplasmic mutations known for arabidopsis belong to the so-called plastomic mutations and barely differ in their phenotypic manifestations from chlorophyllic mutations of the nuclear genes--it is true that these are usually forms with variegated leaves, and not forms possessing the same homogeneous pigmentation (the main genetic distinction between them lies in the matroclinous inheritance of cytoplasmic mutations and an inheritance of mutations of genes quite independent from the direction of the crossings). Consequently, methods of detection (but not of analysis!) of cytoplasmic mutations of arabidopsis do not differ in the least from methods described above used for detection of chlorophyllic mutations of nuclear genes. /64

## Biochemical Mutations

We will only examine methods for detecting biochemical mutations, in the narrow sense of the word, i.e., mutations which are auxotrophic in completely defined metabolites and which react specifically to additions of these metabolites by normalizing their phenotype. For arabidopsis, many other types of mutations are known which may be broadly defined as physiologic and biochemical. These are, for example, mutations sensitive to temperature (Langridge, 1965) or osmotic pressure of the nutritive medium (Langridge, 1958a); mutations defective due to photosynthesis and which are capable of development in media with an admixture of monosaccharides (Langridge, 1958); mutations which are stable under high concentrations of chlorates (Oostindier-Braaksma, 1970; Oostindier-Braaksma, Feenstra, 1972; Laan et al., 1971), or mutations having a heightened sensitivity to the presence of organic matter in the

medium--sensitive to complete media (Redei, Barabas, 1971) or to their separate constituents, e.g., cystine (Redei, 1963). It is quite natural that given such a diversity of phenotypes in this group of mutations, we encounter a situation where every mutation requires its own method of detection. For this reason, their generalized description is impossible to achieve, and concrete methods may be found in the literature just cited, as well as in the review of J. P. Redei (1969).

The detection of auxotrophic biochemical mutations first became possible for the higher plants when Langridge developed methods for mass aseptic test-tube cultivation of arabidopsis in an agar mineral medium of strictly defined composition (Chapter 4). This medium is 'minimal' and to detect biochemical auxotrophic, lethal or semi-lethal mutations in this minimal medium, it is enriched with various admixtures. In order to keep an effective record of biochemical mutations, Langridge used the method of consistent detection of their nutritive requirements which became known in the genetics of microorganisms (Langridge, 1958). The essence of the method consists in a procedure which first studies the reaction of the mutant to complete additives, and then its nutritive requirements are concretized in progressively simpler media up to the individual metabolites. Owing to the toxicity of high concentrations and multicomponent organic additives, as early as during the first stage of detection of biochemical mutations it becomes necessary to subdivide the test metabolites into relatively simple groups which are tolerable to the plants. Langridge, during the first stage, used both extracts from natural products (coconut milk, /65 diffusate from pea seeds, hydrolytic products of nucleic acids derived from yeast), and groups of pure substances (group of 11 vitamins, 6 groups of amino acids having 2-5 amino acids in each). While assembling groups of vitamins and amino acids, with respect to composition and quantity, he was guided by their content in plant tissues and the degree of their toxicity. If some mutant reacted to one of the sets of amino acids or vitamins, this called for a testing of the presence or absence of interaction between the various sets and then the specific requirement of the mutant was revealed on the basis of an economical plan of group tests usually employed for this purpose in biochemical genetics of microorganisms (Lindegren, Lindegren, 1951).

After conducting extensive series of experiments, Langridge isolated--for the first time in highly-developed plants--two biochemical mutations: one auxotrophic on the basis of choline and one on the basis of thiamine (Langridge, 1955, 1958). Langridge's work stimulated experiments on biochemical mutations of arabidopsis in various laboratories. In the course of these experiments, methods were also perfected for detecting biochemical mutations. New formulas were suggested for selective media, in particular a set of pyrimidine and purine bases and a new set of vitamins (Jac-

obs, 1940c).

In obtaining biochemical mutations, we can name three 'bottlenecks' which define the laboriousness of the entire procedure: these center on large expenditures of time for sowing the mass material (auxotrophic biochemical mutations are rare), the problems of early diagnosis of mutants, and eliminating non-mutant sprouts; problems of distinguishing between heterozygotes on the basis of mutations and homozygotic normal plants (since most of the biochemical mutations are lethal or infertile, their propagation often calls for heterozygotic forms). To expedite the mass sowing, it has been proposed to effect it from a diluted suspension of seeds (2.5 seeds per ml) suspended in a 0.1% agar solution; this accelerated the sowing by at least a factor of 10 and improved its uniformity (Redei, 1968). To eliminate non-mutant sprouts, we can germinate the seeds in full media in which the normal sprouts fare much worse than the mutants, and they can be clearly observed as early as the 4th through 7th days (Jacobs, 1965, 1965a). Finally, for some mutations, namely those of pyrimidine insufficiency (the most common), there has been discovered the possibility of sorting the heterozygotes off the normal homozygotic plants in the media using an antagonist of pyrimidine--oxythiamine.

Thus, at the present time methods of detecting biochemical mutations of arabidopsis continue to be perfected, but the basic principle is sustained in the process--their detection in selective media.

## Lethal Mutations /66

Lethal mutations are the most extensive class of mutations known for arabidopsis (as well as for drosophila). In nature and time of appearance, they may be dominant or recessive, haplo- or diplophasic, and the latter are further subdivided into embryonal and post-embryonal.

Here we will examine in greater detail just the embryonal lethals for which A. I. Mueller developed a special detection method (Mueller, 1961a, 1963); but we will first pause briefly on other types of lethal mutations and methods of their detection.

Haplophasic lethals are mutations which cause the lack of viability of gametophytes, leading to partial or complete sterility of plants (Mueller, 1965b; Usmanov, Mueller, 1970). Accordingly, the very method of detection of haplophasic lethals is reduced to the recording of sterility; the A. I. Mueller method (cf. below) is, in this respect, the most convenient for experiments on the arabidopsis. But sterility of plants may be caused by factors other than haplophasic lethals, and for this reason additional studies

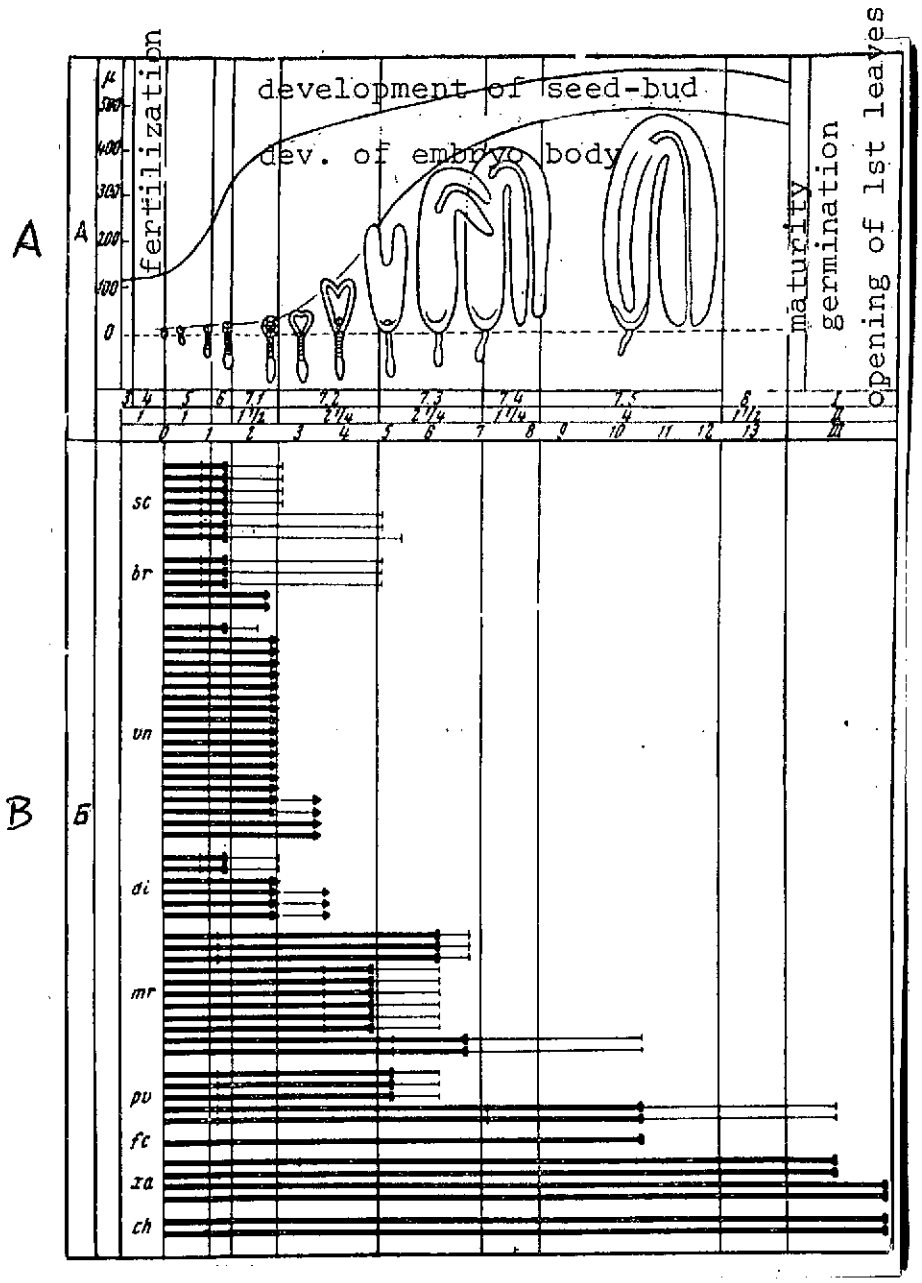



Fig. 12. Embryogenesis in arabidopsis in the norm (A) and classification of embryonal lethals (B) by times of appearance of their lethal effect. (Numbers denote stages of development of embryos (Mueller, 1963)).

/67

are required for their reliable deterination, namely--analysis of heredity and also cytologic study of gametogenesis.

Diplophasic post-embryonal lethals of arabidopsis comprise several morphologic mutations, some chlorophyllic and the majority of biochemical mutations. The methods of detection of these types of mutations have been discussed before. Dominant post-embryonal lethals of arabidopsis are unknown.

Embryonal lethals of arabidopsis are represented by both dominant and recessive mutations. As no distinctions between dominant and recessive embryonic lethals were discovered in the phenotype detection (Mueller, 1965), we will apply the same method detailed below to detect them.

Embryonic lethals of arabidopsis were first discovered by Mueller (1961a) on the basis of studying embryogenesis of arabidopsis in the norm (Mueller, 1961); as a result he was able to isolate a series of sequential stages from the first divisions of the fertilized ovule to the mature seed. While studying the ripening seeds on plants grown from seeds irradiated by X-rays, Mueller discovered splitting (in self-pollination) of embryos which cease their development at certain stages of embryogenesis (Fig. 12). After performing a genetic analysis of the offspring of these plants, he showed that the anomalies observed were hereditary. Mueller then proposed a classification of embryonic lethals based on stages of detection of their lethal action. He isolated a total of seven groups: *sicca, brevis, vana, diffusa, murca, parva* and *fusca*. In addition, as was mentioned before, we can keep track of embryonic lethals by simultaneously discovering chlorophyllic lethal mutations, according to Mueller, of the type *albina, lutea, xantha, chlorina*, since their coatings are colorless and transparent before their seeds ripen and/68 the pigmentation of their cotyledons is clearly visible. According to Mueller's evaluation, the former account for 90% of the sum of embryonal and chlorophyllic lethals.

Great interest is aroused by embryonic lethals when we compare the influence of seed radiation conditions on the somatic and genetic effects of arabidopsis. Indeed, if we can expect some sort of similarity between somatic and genetic effects in their relationship to radiation conditions, then such similarity will be most readily discovered in the case of "mass" mutations.

The technique of recording embryonic lethals was briefly discussed in connection with chlorophyllic mutations. We will examine this problem in greater detail.

The first condition for successful discovery, and all the more so for classification of embryonic lethals, is a correctly chosen recording time. We can consider the optimal period as the time when

the pods are still completely green but the embryos in normal (non-mutant) seeds are already fully developed (Fig. 12). The seed coverings have not yet begun to be impregnated with suberin and acquire a brownish covering. It is precisely at this time that it is easiest to distinguish the embryos that have stopped their development at various stages from normal embryos. According to conditions of the medium and, above all, temperature, this may occur on the 8th to 14th day after the blossom has bloomed. If the recording is conducted very early, the normal embryos are still undeveloped, the seeds are watery, and the cotyledons contain too little chlorophyll. Under these conditions, it becomes difficult to distinguish from the norm both the embryonic and chlorophyllic mutations. If, on the other hand, the recording takes place too late, the seed coatings lose their transparency, enter the stage of pigmentation, and it becomes impossible to distinguish the chlorophyllic mutations from normal. It is true that embryonic lethals are visible even in the event of delayed inspection, as the mutant seed-buds are dehydrated, get wrinkled and brownish, but their classification is impossible since we must squeeze out the embryo from the coating (using gentle pressure of tweezers) and check at what stage its development had stopped--but this can be done while the seed-buds are turgescent. To the point, the impregnation of seeds with suberin begins earlier for mutant seeds than for normal seeds; the earlier it begins, the earlier the embryo dies. On this basis, the distinction between sterile seed-buds and embryonal lethals which are typical of the very early stages of embryogenesis is founded. By size and shape they are sometimes barely discernable, but sterile seed-buds retain the appearance of small white lumps until the pods ripen; but the early lethals get pigmented very quickly and at times, intensely.

We can see from Fig. 12 that with the majority of embryonic lethals, the time of destruction of the embryos varies quite widely. For this reason, classification of embryonic lethals is a difficult task. The ideal situation of this classification would involve isolating discrete mutations into heterozygotic lines and studying the variability of phenotypic appearance of each of them individually, a thing that is not always possible and expedient. We noted before that the distinction between embryonal lethals and chlorophyllic mutations in combined recording using Mueller's method also suffers from some uncertainties. In this respect (and according to the problems of the experiment), it is sometimes advisable to record only the total sum of embryonal lethals and chlorophyllic mutations. This particularly applies to experiments on mutagenesis in which the researcher is mainly interested in the overall effectiveness of the mutagenic influence applied, and not in the interrelationship of various classes of mutations according to the dose of mutagen and the experimental conditions. /69

In concluding this section, let us examine the problem of spontaneous background of embryonic lethals and the non-inherited anomalies of embryogenesis of arabidopsis. According to Mueller (1963 et passim), the level of spontaneous embryonic lethals is very low--on the order of one-tenths or one-hundredths of one percent, and the portion of non-hereditary embryonic anomalies is negligible; moreover, the level of spontaneous lethals is subject to selection. But it is not always possible to attain a low spontaneous level. Under actual experimental conditions, especially in cultivating arabidopsis in open soil or hot-houses, the level of spontaneous anomalies may fluctuate within very wide limits from the previously cited values up to 10 or even 20%. Particularly unfavorable for embryogenesis of arabidopsis are high temperatures when accompanied by insufficient air humidity--this raises sharply both the level of sterility and embryonic mortality. Another cause for the emergence of phenocopies of embryonic lethals may be the injuries done to the plants by pests, particularly by aphids. But phenocopies may quite possibly originate in trauma. The latter is a rather common occurrence in test-tube cultivation, where in the confined space of the test-tube, the pods are crumpled and even twisted (Fig. 6). This damage can be avoided if, during the blooming of the plants, the stoppers are removed from the test-tubes and the pods are allowed to grow freely. It is particularly important to eliminate all causes of the rise of non-inherited embryonic lethals as such non-inherited anomalies may change the essential relationship of classes in the split offspring. As concerns mutagenic experiments, although the heightened background of embryonic anomalies does reduce the exactitude of obtained results, the causes of background heightening affect the control and experimental plants in an equal (or similar) manner and consequently, cannot inject substantial distortions into the results.

## Chapter 8. Methods of Genetic Research. Quantitative Analysis of Mutations

### 8.1. General Remarks /70

In examining methods of quantitative analysis of mutations in arabidopsis, we will limit ourselves to a discussion mainly of methods of quantitative analysis of induced mutations occurring in mutagenic action on seeds.

This limitation is governed by the fact that methods of quantitative analysis of mutations induced in the seeds of self-pollinating plants have a number of specific peculiarities distinguishing them from, say, general quantitative methods of genetic analysis which are applied equally to all living organisms. Moreover, these general methods are well developed in special textbooks (Mather, 1957; Weber, 1967; Serebrovskiy, 1970, etc.).

Methods of quantitative analysis in plants of mutations induced by mutagenic action on the gametes have no substantial peculiarities (usually the pollen. Perhaps only during the quantitative analysis of the results of the appropriate crossings do we have to take into consideration that mnay mutations of plants occur with a frequency lower than expected. Thus, for example, in cases of mono-hybrid crossings, the frequency of mutations may be 25% lower than expected. This situation is also observed in arabidopsis. Thus, the mean frequency of recessive embryonic lethals in mono-hybrid crossings amounts to 20-21% (Mueller, 1963) and the mean frequency of chlorophyllic mutations (recorded in sprouts in the cotyledon phase) amounts ot 18-19% (Ivanov, 1971). Such deviations are essential above all to determine the volume of selections needed for distinction between the various possible relationships of classes. The cause of a "shortage" of mutants, as it also happens with other organisms, derived from a lowered vital capacity of homo- or hemizygotes in the mutations which can be found in various phases of the cycle, but mainly in the stage of the gametophyte, during the germination of the pollen tubes (Buchholz, Blakeslee, 1930, 1936) as well as in germination of seeds (Ivanov, 1971).

The situation becomes complicated when the mutagenic impact affects plant seeds, which most often occurs. But among the advantages of mutagenic treatment of seeds are the possibility of variation of experimental conditions within broad limits, the possibility of applying the most diverse and, at times, very "rigid" supplementary effects, the possible comparison of somatic and genetic effects, etc.

However, in the process of mutagenic influence on the plant

seeds, there emerges a far from simple task--how to determine a quantitative relationship between the frequency of mutations which are being recorded and the frequency of mutations induced in the meristemic cells of the embryos. In order to get an idea of the complexity of this problem, let us consider the general plan of the embryo structure of an arabidopsis seed and the formation from it of the basic organs of a fully-matured plant. Data required for such analysis are available in several published works (Vaughan, 1952, 1955; Vaughan, Jones, 1953; Miksche, Brown, 1963, 1965; Mueller, 1963). /71

The embryo of a ripened seed of arabidopsis consists of a hypocotyl with two fully-developed cotyledons facing each other at the upper end and a primary rootlet at the lower end. The primary rootlet has a root meristem at its end and between the cotyledons is an apical meristem which is of primary interest to us. The apical meristem consists of a great many cells (on the order of thousands) which form anatomically distinct body and tunic. In the germination process, in the next stage of development, the greater part of the cells of the apical meristem of the embryo participates in the formation of vegetative organs and just a small portion of its cells located in the second layer of tunic are the initial cells of the reproductive origin leading to the development of flowers--the reproductive organs of the plants. In the corresponding parts of the flowers there are formed haploid formations--male and female gametophytes (as a result of micro- and megasporogenesis); this in turn leads to the formation of haploid sperms and ovules. Finally, as a result of self-pollination within the boundaries of each blossom and the subsequent fertilization, there arises a new diploid generation. For the purpose of the problem discussed here, it is essential that the fruits of arabidopsis are multiseeded and that all the seeds of one pod emerge as a result of self-pollination within the boundaries of a single blossom.

Mutations induced in arabidopsis by mutagenic action on the seeds are, as a rule, recessive (at any rate, up to now, we did not yield one dominant mutation) and they come up habitually in a heterozygotic state since a simultaneous appearance of identical mutations in homologous chromosomes of some initial cell is a most unlikely event. Consequently, mutations which occurred in a seed embryo are not detected phenotypically either in the initial cells or in the reproductive organs derived from them; they can only be detected in the $M_2$ generation, when some of them are homozygotized due to self-pollination. Naturally, this principle does not extend to those chromosomic or genomic mutations which may be detected cytologically.

Thus, there is a long and complex chain of ontogeny between the induction of mutations in seed embryos and their appearance; and consequently, between that time and their analysis in the di-

plophase of the next generation.

The unknown quantity characterizing the effectiveness of /72
mutagenic effects is the number of mutations induced in initial cells of the reproductive meristem while said quantity is the number of mutations which appeared in the next diplophase. The problem is to find the quantitative correlation between these two quantities. From what has already been said, it is clear that this is not a simple task. Its resolution requires the possession of some quantitative estimation of the yield of mutations in $M_2$, as well as direct data, or at the very least, scientific assumptions on the structure of the reproductive meristem of the seed embryo (the number of cells and the distribution of mutations among them), the relative viability of mutation-free cells and cells containing mutations, the methods of separation of blossom backings from the general mass of cells in the reproductive meristem (more precisely their reproductive parts), the system of crossings within each self-pollinating flower, the nature of inheritance of induced mutations, and finally, the relative fertility of mutation-free and mutation-bearing plants and pods. Let us now begin to examine these problems.

## 8.2. Registering indices of induced mutation

For a quantitative estimation of the yield of induced mutations in the $M_2$-generation, in the genetics of plants we use three parameters: $m_a$--frequency of plants $M_1$ in whose $M_2$ offspring the mutants of the registered class are splintering; $m_b$--the frequency of fruits M among whose seeds occur the mutants of the class being registered; $m_c$--the frequency of mutants of the reigstered class in the M2 generation.

These same definitions, upon introducing the notation required for further exposition, can be written as follows:

$$m_a = \frac{A_m}{A_m + A_0} = \frac{A_m}{A}, \quad (1)$$

$$m_b = \frac{B_m}{B_m + B_0} = \frac{B_m}{B}, \quad (2)$$

$$m_c = \frac{C_m}{C_m + C_0} = \frac{C_m}{C}, \quad (3)$$

where A--the number of plants M1; B--the number of fruits(pods) in M1; C--the number of individuals in M2 and the subscripts m and o denote the numbers, respectively, for A--of splintering and non-splintering families in M1; for B--of fruits(pods) among whose seeds there are or are not mutants; and for C--mutant and /73

non-mutant individuals in M2. It is also clear that A, B and C without subscripts denote the total numbers of plants in M1, pods in M1 and plants in M2, respectively.

The basic properties of these three parameters of the appearance of mutations in the M2-generation have already been discussed in detail in the literature (Gaul, 1960; Mueller, 1965;, Li, Redei, 1969).

Let us begin with their methodologic distinctions. To define $m_a$ we will require a family-by-family analysis of the splintering in the M2-generation; to define $m_b$--an analysis of seeds from each fruit in M1 individually, whereas to obtain the definition of $m_c$, we need to know the total numbers of mutants and non-mutants of the M2-generation. Ths the definition of $m_c$ appears to be the least labor-consuming and, in this respect, the most convenient one, provided the purposes of the investigation do not require a genetic analysis of mutations which had appeared in succeeding generations.

What then is the connection between the quantities $m_a$, $m_b$ and $m_c$ with the unknown quantity--the average number of induced mutations per initial cell of the reproductive meristem (let us denote this quantity by M). Let us mention, by the way, that S. L. Li and J. P. Redei (1969) prefer to express the effectiveness of the mutagenic effect with the quantity R--by the mean number of induced mutations per genome per unit dose D, postulating correctly that the quantity R is more convenient to compare the effectiveness of mutagenesis in organisms of varying ploidity. But by virtue of the obviousness of the simple equation

$$R = \frac{M}{PD}, \qquad (4)$$

where P represents the ploidity of cells subjected to mutagenic effects, the distinctions between R and M are not important. At the same time, the quantity M is much more convenient in constructing general models connecting $m_a$, $m_b$, and $m_c$ to the number of induced mutations.

It is also self-evident that the average number of mutations per cell (M) characterizes much better the mutagenesis than does the frequency of cells with mutations (m) which does not account for the number of mutations occurring in the cell, although this index is quite frequently used in plant genetics, e.g., in the experiments on arabidopsis (Mueller, 1964a and other works). Consequently, in our future explanations we will just make use of the quantity M.

But let us return to the connection between $m_a$, $m_b$, and $m_c$ to M. At low frequencies of mutation (e.g., when mutations of quite

definite loci are registered or when the doses of mutagenic effects are very small) it is quite permissible to assume that the preponderant majority of the appearing mutations are distributed in a quantity of one per embryo, and that the cases are very rare where one embryo containing a limited number of initial cells will have two or more rare mutations. In that case it is easy to find the connection between M and $m_a$, provided the number of initial cells in the embryo (n) is known, namely /74

$$M = \frac{m_a}{n} \qquad (5)$$

This ratio (under an assumption that n = 2, which is substantiated below), lies at the base of methods of evaluation of the number of induced mutations by J. Langridge (1958) and Li and Redei (1969). But the former introduces a correction regarding the effectiveness of recording of mutations in specimens of a specific volume, while the latter express the effectiveness of mutagenesis using the previously noted quantity R (4).

With an increase in the dose of mutagen, and in recording frequent mutations, the probability increases that more than one mutation is induced in initial cells of one embryo. In case such independently emerging mutations are phenotypically indistinguishable, they will be registered as one mutation, and the evaluation of M on the basis of ratio (5) will be lowered; and the stronger it will be the larger the dose of mutagen or the more frequently the mutations of the registered class occur. For example, if the number of mutations in the initial cells follows the Poissonian distribution, then when M = 0.5 mutations per cell the frequency of cells containing more than one mutation would be 10%; if M = 1, the frequency will be 26%. Thus the methods of Langridge as well as those of Li and Redëi work quite well for rare mutations and small mutagen doses; they are also quite suitable for "average" doses and mutation frequencies (say if $M \leq 0.5$), provided that the various mutations which had formed independently in the cells of one plant as well as their combinations are phenotypically distinctive. In the opposite situation, it becomes necessary to have other relationships between M and $m_a$.

The quantity $m_a$ has yet another feature which makes it poorly suited for large mutagen doses, namely: the dependence of $m_a$ on the dose (especially with chemical mutagens but also in irradiation), which is depicted by a steeply rising S-shaped curve which, in specimens of ordinary volume (on the order of several hundreds or thousands of plants in M1) quickly reaches a 100% effect even at doses where some of the plants are still viable and sufficiently fertile; and the quantities $m_b$ and $m_c$ in particular continue to increase.

Sometimes noted as a merit of the quantity $m_a$ (e.g. by Li, Redei, 1969) is the fact that in contrast to $m_c$ it does not depend on deviations in any direction from the Mendelian relationships. But these same authors use a quantity similar in its properties to the $m_c$ and namely, the frequency of mutants in the splintering families of M1 to evaluate the number of initial cells in the seed embryos. And this, in turn, lies at the base of their method of recording induced mutations. We will yet return to deviation from Mendelian relationships when we discuss $m_c$.

The quantity $m_b$ is in many respects similar in its properties to $m_a$, but according to the previously cited definition, /75 the unit of registration in this case is not the entire plant M1, but the individual multi-seed fruit. This can also be an individual collective fruit, e.g., in the case of cereals--a single ear (Gaul, 1960). Consequently, all that has been said about the limitations of the application of $m_a$, under the conditions of increasing doses of mutagens and of frequency of mutations, applies likewise to $m_b$, but all complications in this case manifest themselves under higher doses and mutation frequencies. In experiments on arabidopsis, the circumstance which argues in favor of the use of $m_b$ is one under which, apparently, individual flowers within whose boundaries the self-pollination is effected, in plants chimerical in their mutations are, as a rule, non-chimerical, i.e., all the progamete cells of the same flower have the same genotype (Mueller, 1964a).

For this reason, if the cells of a flower are heterozygotic on the basis of mutations, then within the bounds of the fruit which is formed from it, we will observe the usual splintering not complicated by the uncertainty of frequencies of the gametes of various classes which would have been the case in chimerical flowers. The hypothesis on the genetic homogeneity of arabidopsis flowers permits us to find a relationship between $m_b$ and M without having recourse to data on the number of initial cells in the seed embryo (Mueller, 1965f). But, quite aside from inconveniences common to $m_a$, the quantity $m_b$ also has one specific deficiency--the inevitability of working with small specimens. Indeed, the number of seeds in an individual pod of arabidopsis may vary from several units to several dozens; and with any mutagenic effect the number drops in proportion to the dose so that we must operate with 10-20 seeds, sometimes even less. A. I. Mueller (1964a), in defining $m_b$, takes into account pods containing no less than 4 seeds. It is easy to prove that if the progamete tissues of the flower are heterozygotic based on one mutation and the anticipated frequency of recessives $f = 0.25$, then the probability of the mutant splintering in a 4-seed pod is less than 0.7; with a usual deficit of recessives ($f \approx 0.2$) this probability is even lower.

The quantity $m_c$--the frequency of mutants in the M2-generation --is free of the deficiencies inherent in the quantity $m_a$. In the first place, it comprises by definition all emerging mutations (in contrast to $m_a$); in the second place, in the process of defining $m_c$, the entire crop of plants M1 or its representative part (in contrast to $m_b$) is taken into account; and in the third place, along with the growth of the dosage or mutation frequency, $m_c$ increases much more slowly than $m_a$ or even $m_b$, so that even under conditions of sub-sterilizing doses of all mutagens studied on arabidossis, the value of $m_c$ is still far removed from 100%. This is due to the fact that each of the incipient recessive mutations is spearated from the heterozygotes with a relatively small frequency of f = 0.2. Since in the K-hybrid crossing, the frequency of a dominant (nonmutant) phenotype is $(1 - f)^d$, even with an improbably large average number of mutations per cell, e.g. M = 5 (one mutation for each pair of chromosomes), the frequency of nonmutant individuals in M2 would still amount to $(1 - f)^5 = 0.33$, or 33%.

Thus, of the three parameters of mutagenic effectiveness in /76 the case of plants--$m_a$, $m_b$, and $m_c$--the latter can be considered universal. And the objection raised before that $m_c$ is highly dependent on deviations from the Mendelian relationships (which may vary greatly according to the genotype of the plants and the conditions of their growth: Li, Redei, 1969) is indeed essential in recording rare mutations and in working with small specimens (but $m_a$ is used for that purpose). As to the quantitative recording of the vast classes of mutations and in working on large specimens, it follows that under such conditions, despite considerable fluctuations in the frequency of mutant splintering from heterozygotes (f), its average value remains quite stable. Thus, in studying the successive generations for splintering in offspring of heterozygotes of 60 different embryonic lethals and chlorophyllic mutations calculated in the pods of the maternal plants, the average value of f was 21% (Mueller, 1963); and an identical value for 26 independent chlorophyllic mutations in the sprouts gave f = 18% (Ivanov, 1971). We can venture a guess that in using both methods, the average frequency of splintering of recessives from the heterozygotes is f = 20% based on embryonic and chlorophyllic mutations.

In analyzing the applicability of the parameters $m_a$, $m_b$ and $m_c$ to study the mutagenesis in tests on barley and using somewhat different arguments, Gaul arrived at a conclusion that the most suitable parameter is provided by m (Gaul, 1959). This opinion is also held by Mueller with respect to arabidopsis (Mueller, 1965). But this categorical tone is barely warranted since if we do not merely have to record mutations but must isolate them and study them further, the parameter $m_a$(frequency of plants producing a splintering offspring) is much easier to use. It

is quite a different matter that in conversion we should be aware of the limitation of the applicability of formula (5) and use other methods which will be discussed later.

To convert from the recorded parameters to the number of individual mutations M, we must also have some data on the structure of chimerical plants which can be wholly characterized as data on the "embryonic path" in arabidopsis.

## 8.3. The Structure of chimerical plants

Before going any further, we will return to the fact that an embryo of a seed is a mutilcellular formation, and the nucleus of each cell contains many genes capable of mutations in a variety of directions. Consequently, there is scant probability that any applied mutagen (and all the more so for an unspecific one as ionizing radiation) would call forth in all meristemic embryonic cells identical mutations. And this means that as a result of mutagenic action, the meristem of the seed embryo becomes chimerical, i.e., it consists of cells of various genotypes. The only meristems which will be virtually nonchimerical will be those whose cells do not elicit any mutations. In the event that the apical meristem of the seed embryo has future reproductive tissue represented by more than one initial cell, this tissue too will generally be chimerical--this is indeed observed in arabidopsis (Mueller, 1965, 1965e; Van der Veen, Gerlach, 1965); it is also seen in other plants (Gaul, 1960; D'Amato, 1965). The number of initial cells, however, must be relatively small. This is also proven by the experiments of T. Fujii and those of Gichner and Veleminsky on the subject of induced somatic mosaicism (Fujii, 1964; Gichner, Veleminsky, 1965). Fujii, in the process of irradiating dormant arabidopsis seeds which were heterozygotic on the basis of a recessive mutation gb' (glabrous'), obtained 80 mosaics with surface dots on the rosette leaves; he did not succeed in observing mosaic dots on the floriferous stem, even though the gene gb' does control its release. Gichner and Veleminsky studied induced somatic mosaicism on seeds which were heterozygotic on the basis of their chlorophyllic mutations albina, xantha and chlorina, exerting influence on them by chemical mutagens and X-rays. In the process, they obtained 406 different mosaics, with the dots generally distributed on the rosette leaves. They observed dots extending to the floriferous stem, but without citing the figures, the authors indicate that this as a rare occurrence. The rare occurrence of dots on stems as compared to the rosettes indicates that in the apical meristem of a seed embryo, future tissues of the stem are represented by a relatively small number of cells.

But we could go still further and attempt to 'count' the initial cells, using data on splintering in their offspring of

chimerical plants. This was first done by Langridge (1958), who discovered that the average ratio of phenotypically normal plants to mutants in an offspring of 24 chimeras of different mutation amounted to about 7:1, while in subsequent generations the heterozygotes of all these mutations had produced the customary monohybride splintering of 3:1 or something very close to it; thus the ratio 7:1 could not be explained by the greatly diminished viability of homozygotes of recessive mutations. Further, on the basis of their inheritance in subsequent generations we could maintain that in each of the 24 families studied the splintering occurred only in one mutation. But then the ratio 7:1, being too large for splintering in the offspring of a nonchimeric heterozygote, is too small for splintering in the offspring of a plant which is chimeric on the basis of only one mutation and forms reproductive organs from many initial cells. This high frequency of recessives in the offspring of a chimera is only possible in one case, namely: if the number of initial cells is two, one of /78 which is nonmutant and the other is heterozygotic in mutation; if these cells breed with roughly the same rate, this produces *in toto* about the same number of seeds in each clone; and if, in cross-breeding the gametes stemming from different initial cells do not mingle then for each 4 normal descendants of one initial cell another one yields 3 normal ones and 1 mutant, i.e., the ratio will be 7:1. And if in crossing gametes derived from two such cells there takes place a *panmixia*, the ratio will rise to 15:1. In the event of *partial* intermixing of gametes, the ratio of phenotypically normal and mutant individuals among the chimeral offspring will assume intermediate values between 7:1 and 15:1. If the number of initial cells is greater than two in the presence of one mutation, the ratio of phenotypes t:mut increases even more: in the event of isolation between gametes descended from different initial cells, it is described by the general formula $(4n - 1):1$, where n is the number of initial cells (Li, Redei, 1969), and for panmixia by $(4n^2 - 1):1$. Consequently, Langridge's assumption about the average number of initial cells in arabidopsis being equal to two is quite probable. But he also had occasion to note that the number of initial cells varies considerably: in specific chimeras he found ratios of t:mut which corresponded to numbers of initial cells from 1 to 5. Partially larger ratios could, of course, be caused by incomplete isolation between gametes of various derivations faced with a small number of initial cells. Values approaching two of the average number of initial cells in seed embryos of arabidopsis were obtained more than once in independent experiments of various authors. Thus, in our studies of splintering in offspring of 268 radiation chimeras on the basis of chlorophyllic and morphologic mutations, we obtained an average ratio of 8.9:1 which corresponds to $n = 2.5$ (Nikolov, 1968; Nikolov, Ivanov, 1969). In the experiments of Li and Redei dealing with descendants of 182 radiation chimeras based on chlorophyllic mutations

which they denoted as 'pale seedlings' they obtained an average ratio of phenotypes t:mut = 5.9:1 (n = 1.7) and in the offspring of 370 EMS chimeras in the same mutation--4.4:1, which gives n = 1.5 (Li, Redei, 1969). In our other experiments, the average ratio of phenotypes in offspring of 26 radiation chimeras in chlorophyllic mutation albina, xantha, chlorina and viridis registered in sprouts of M2 amounted to 7.8:1, which gives n = 2.2 (Ivanov, 1971); and in the offspring of 37 chimeras in chlorophyllic mutations albina, xantha and chlorina registered in pods in M1 it ran to 6.9:1 (n = 2.0). It is true that in these experiments the ratio of phenotypes varied with the different doses of seed irradiation and no definitely pronounced dose tendency could be traced in the process (Tables 4 and 5).

TABLE 4 SPLINTERING IN OFFSPRING OF CHIMERAS IN CHLOROPHYLLIC MUTATIONS REGISTERED AMONG SPROUTS OF M 2 GENERATION /79

| Dose of gamma-radiation, krad | 10 | 20 | 40 | 70 | 110 | Total |
|---|---|---|---|---|---|---|
| Number of normal shoots | 488 | 556 | 301 | 326 | 63 | 1734 |
| Number of mutants | 46 | 79 | 29 | 62 | 7 | 223 |
| Their ratio | 10.6:1 | 7:1 | 10.4:1 | 5.3:1 | 9:1 | 7.8:1 |

TABLE 5 SPLINTERING IN OFFSPRING OF CHIMERAS IN CHLOROPHYLLIC MUTATIONS REGISTERED IN PODS OF M 1 GENERATION

| Parameter | X-ray dose, krad | | | | | Neutrons, 5.6krad | | TOTAL |
|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 5.2 | 10.5 | 15.6 | |
| Number of normal embryos | 142 | 490 | 922 | 553 | 590 | 164 | 13 | 2874 |
| Number of mutants | 25 | 45 | 123 | 99 | 85 | 36 | 2 | 415 |
| Their ratio | 5.7:1 | 10.9:1 | 7.5:1 | 5.6:1 | 6.9:1 | 4.6:1 | 6.5:1 | 6.9:1 |
| Number of pods with mutant embryos | 2 | 8 | 23 | 26 | 17 | 18 | 2 | 96 |
| Total pods | 5 | 20 | 44 | 48 | 33 | 25 | 4 | 179 |
| Relative width of mutant sector, % | 40 | 40 | 52 | 54 | 52 | 72 | 50 | 54 |
| Number of normal embryos in pods with mutations | 49 | 150 | 484 | 266 | 297 | 115 | 4 | 1368 |
| Number of mutant embryos in them | 25 | 45 | 123 | 99 | 85 | 36 | 2 | 415 |
| Their ratio | 2.0:1 | 3.5:1 | 3.9:1 | 2.6:1 | 3.5:1 | 3.2:1 | 2:1 | 3.3:1 |

Finally, in the experiments of Relichova (1972), as a result of induction of the viable chlorophyllic and morphologic mutations /

by x-rays, ethylmethanesulfonate and nitrosomethyl urea, there was likewise obtained the best correspondence to the anticipated (n being equal to 2) ratio of normal and mutant individuals, 7:1.

Of course, the value of n = 2 obtained in these experiments cannot be regarded as a "world constant", as in addition to the variations in specific plants already noted, this magnitude also depends on the conditions of the experiments. For instance, if we artificially delay the switch of plants M1 to a generative development period (a short day), then the number of cells in the reproductive meristem, estimated on the basis of splintering data, will increase (Mueller, 1965; Li, Redei, 1969). /80

However, Mueller is wrong, too, when he considers that the varied ratios of phenotypes precludes the possibility of its use for an estimate of the number of initial cells (Mueller, 1965). Moreover, he advances the following arguments: in the first place, according to his data, (1964a), with an increase in the dose of mutagen of nitrosomethyl urea the ratio of phenotypes decreases smoothly and the relative width of the mutant sector in the case of chimeric plants, calculated as the ratio of the number of pods with mutants to the total number of pods, increases; and in the second place, in specific chimeric plants the relative width of the mutant sector changes along the axis of the main collective fruit (he was comparing 1-5th pods with the 21-25th ones): it can either decrease or increase so that its chimerical quality may be altogether lost. However, according to his own data (Mueller, 1965), the <u>average</u> width of the mutant sector, calculated for a specimen from among the 255 chimeras, <u>does not change</u> in the process. But then on the basis of ratios of phenotypes in the offspring of chimeras, only the average number of individual cells is defined. And in addition, knowing the variation of this number, we must remember that the estimate is suitable only under well-defined conditions. Moreover, the cited experiments of Mueller, in methodologic respects, do not quite correspond to the problem which has been posed. Indeed, all his estimates were made for the sum of embryonic and chlorophyllic details and this, as was noted earlier, is the vastest class of mutations in arabidopsis; as such it is ill suited for the study of the structure of chimeras--the results are greatly exaggerated due to simultaneous splintering of different mutations. Consequently, the results are much more reliable when they are obtained in experiments with relatively rare mutations for which the probability of simultaneous appearance of more than one mutation in the embryo is small. In such experiments (Table 5), the relative width of the mutant sector in chimeric plants of M1 proves to be near 50% (which is expected when n = 2). It is not dose-dependent.

Let us make two concluding observations on the number of initial cells. As will be seen from what follows, in calculating the average number of induced mutations (M), an estimate of the number of initial cells is needed only if the registering parameter is provided by the frequency of splintering families of the M1 generation, i.e., $m_a$. And the last thing: the small number of initial cells calculated for the apical meristem of the seed embryos of arabidopsis is near to the results of similar estimates for certain cereals: n = 1-4 (Gaul, 1964, 1965; D'Amato, 1965).

Let us now address ourselves to the problem of the distribution of mutations by individual cells. Since mutations are rare and independent events and the probability of occurrence of a mutation in each specific cell is small, and the total number of initial cells (in the entire embryo complex) is great, it seems /81 quite likely to assume that the number of mutations in the initial cells, in general situations, follows the Poissonian distribution. In a specific case, when the frequency of mutations is small and the share of cells containing two or more mutations is correspondingly small, we can assume an approximate basis that no cell can have more than one mutation.

In this case, it is easy to demonstrate that in the absence of an ontogenic solution and if the flowers are not chimeric and panmixia does not occur within each of them in self-pollination, the average number of mutations per cell (M) or (what here amounts to the same thing numerically) the frequency of mutant cells (m) are linked to the frequency of mutants in M2 by a very simple correlation, namely:

$$m_c = f \cdot m = f \cdot M$$

with the notations used above. This formula was offered by O. Frydenberg for evaluating mutagenesis in barley (1963). Given low doses of mutagens and in rare mutations, all the basic conditions of its application are fulfilled in experiments on arabidopsis. But it is unsuitable in registering embryonic lethals when the assumption of mutation distribution of no more than one per cell is unlikely.

Mutations which occurred in cells may be subjected to the influence of ontogenic selection and this would bring about distortions of the mutation frequencies (Langridge, 1958; Gaul, 1965). In all probability, the distinction in the relative viability of cells (normal and heterozygotic in mutations) may be both large and small, positive and negative and may be absent altogether (Mueller, 1965). But the effect of this factor on the registered frequency of mutations seems impossible to evaluate quantitatively. Consequently, we must simple remember that in

defining the frequency of mutations, we can recognize it only with precision up to the pressure of ontogenic selection.

In constructing models to connect the reigstered frequencies of mutants to the number of induced mutations in self-pollination, it is extremely important to know whether or not the flowers where self-pollination is effected are chimeras. For a judgment on this matter, let us turn to the data given on the bottom line of Table 5, where the average ratio of phenotypes in pods M1 containing mutant embryos M2 are given. It is seen from the table that the total ratio of phenotypes conforms well with the anticipated 3:1 ratio under the conditions of monohybrid splintering (with the usual moderate shortage of recessives) and the observed deviations between the versions of irradiation are explainable by the paucity of specimens. Such a ratio can be observed only in the case where tissues of the flowers are non-chimeric. This fact is also attested by similar ratios obtained by Mueller (1964a) for the sum of embryonic and chlorophyllic mutations with the only difference being that in his experiments with nitrosomethyl urea, the ratio (near 3:1) /82 was observed only at the minimum dose of mutagen; with an increase in the dose, it decreased to 0.7:1, a factor which is linked to an increase of mutations per cell. Of course, even in this case, the data of mass mutations are less convincing than in rare mutations, since ratios that are similar to 3:1 (and less) may be obtained in self-pollination in chimeric flowers if the overall number of mutations is sufficiently large. As to the data cited in Table 5, these have reference to solitary chlorophyllic mutations, and for this reason, the conclusion about the nonchimeric nature is deemed to be valid. The ratios close to Mendelian ones also indicate the fact that in self-pollination there occurs panmixia within the limits of the flowers.

Where the number of mutations in a cell may be greater than one, it is also important how they splinter in cross-breeding. For example, we know about the embryonic and chlorophyllic mutations of arabidopsis (Mueller, 1964a) whose double, triple, etc. recessives are phenotypically indistinguishable and cannot be distinguished from simple recessives without a special genetic analysis. Consequently, we can assume that a zygote yields a mutant phenotype (not identified closer than that) if at least one mutation is homozygotic and this means that without calculating coupling when the number of mutations in the progametic cells of the flower is equal to k, the frequency of nonmutant zygotes will be $(1 - f^k)^k$, where f is the portion of mutants splintering out in monohybrid crossing up to each one of the k mutations.

And, finally, the last question which is partially associated with the previously mentioned ontogenic selection, namely: do chimeric plants differ from nonchimeric ones with respect to

fertility, and also do pods with mutations differ from the 'normal' pods? The comparison that were made with respect to both the chlorophyllic mutations and their sum with embryonic lethals showed that at any doses of irradiation, the fertility of chimeric and nonchimeric plants, as well as the number of seeds in the pods with or without mutations are not authentically distinguishable.

Arriving at a grand total of registered quantitative characteristics of an induced mutational process in plants $m_a$, $m_b$ and $m_c$, as well as of what can be termed in a general form 'the structure of the embryonic path' we will formulate the basic premises for constructing a model linking these registered quantities with the average number of induced mutations per initial cell of the reproductive meristem.

a. Out of the three quantitative characteristics of an induced mutational process in plants, namely the frequency of plants M1 which yield a splintered offspring ($m_a$); the frequency of fruits (or specific collective fruits) that contain mutations in plants M1 ($m_b$) and the frequency of mutants in M2 ($m_c$)--the latter is most universal and particularly good in experiments dealing with mutagenesis; while the first one is good in recording rare mutations, particularly when isolation and analysis of specific mutations is /83 required, and the second one occupies an intermediary position.

b. The number of initial cells of a reproductive meristem in the seed embryo (n) is small and may be defined by genetic methods with sufficient precision to meet the specific conditions of the experiment. As a rule, $n > 1$, the fact that conditions the chimeric nature of plants in M1.

c. Mutations occurring in mutagenic action on the seeds are generally recessive, and their number in initial cells follows the Poissonian distribution curve.

d. The mutations which appear may be subject to the action of ontogenic selection, but this action is not susceptible to quantitative evaluation and cannot be accounted for in simulation. The effects of ontogenic selection do not affect plant fertility.

e. Flowers within which self-pollination and panmixic cross breeding occur are not chimeric, but consist of cells of a single genotype. This prerequisite is very significant singe it allows us to regard the offspring of each initial cell and then to sum up the results in all the cells.

f. Zygotes containing in their homozygotic state at least one mutation of the registered class are phenotypically mutant.

## 8.4. Relationship between recorded frequencies $m_a$, $m_b$, $m_c$ and the number of induced mutations M

Let us recall the notation introduced earlier: A, $A_0$, $A_m$--the number of plants in M1; B, $B_0$, $B_m$--the number of pods in M1; C, $C_0$, $C_m$ --the number of seeds in M1 (= individuals of M2) where the subscripts o and m indicate the nonmutant and mutant plants, pods or seeds, respectively; n is the number of initial cells in the embryo; M is the average number of mutations per initial cell; f is the average frequency of splintering of mutants from the heterozygotes.

Let us also denote the average fertility of plants in M1:

$$S = \frac{C}{A} = \frac{C_0}{A_0} = \frac{C_m}{A_m}.$$

Let us begin with a more general model linking M to $m_c$ and we will illustrate its structure in Table 6.

TABLE 6. CONSTRUCTING A MODEL TO ESTABLISH A LINK BETWEEN THE AVERAGE NUMBER OF INDUCED MUTATIONS IN INITIAL CELLS AND THE FREQUENCY OF MUTANTS IN THE M2 GENERATION

| Number of mutations in initial cell | Number of initial cells with mutations | Number of nonmutant zygotes in M2 | Number of mutant zygotes in M2 |
|---|---|---|---|
| 0 | $n_0 = Ane^{-M}$ | $\frac{n_0}{n} \cdot S$ | 0 |
| 1 | $n_1 = AnMe^{-M}$ | $\frac{n_1}{n} \cdot S(1-f)$ | $\frac{n_1}{n} \cdot S[1-(1-f)]$ |
| 2 | $n_2 = An\frac{M^2}{2!}e^{-M}$ | $\frac{n_2}{n} \cdot S(1-f)^2$ | $\frac{n_2}{n} \cdot S[1-(1-f)^2]$ |
| ... | ... | ... | ... |
| k | $n_k = An\frac{M^k}{k!}e^{-M}$ | $\frac{n_k}{n} \cdot S(1-f)^k$ | $\frac{n_k}{n} \cdot S[1-(1-f)^k]$ |
| $\sum_{k=0}^{\infty}$ | $An$ | $C_0 = ASe^{-fM}$ | $C_m = AS(1-e^{-fM})$ |

Thus, it follows from the table that:

$$m_c = \frac{C_m}{C_0 + C_m} = 1 - e^{-fM} \qquad (7)$$

or

$$M = -\frac{\ln(1 - m_c)}{f}. \qquad (8)$$

From formula (8) it can be seen that the ratio of M and $m_c$ does not depend on the number of initial cells, the only condition being that the average number n be the same in both chimeric and nonchimeric plants.

Besides, formula (8) is only valid under the conditions that /84 all the prerequisites formulated in the previous section of this chapter are equally correct.

This formulas was first proposed, wtihout conclusion and analysis, by A. I. Mueller (1965). But he simplified the situation too much in asserting that the only necessary prerequisite for its application is the Poissonian distribution of mutations in initial cells.

Now it will not be difficult to establish the correlation between M and $m_b$ as well as between M and $m_a$.

Indeed, if every initial cell is the ancestor of an average of b nonchimeric pods, the number of pods without mutations is $B_0 = n_0 b$, which in referring to the first line of Table 6, may be written in the form $B_0 = Anbe^{-M}$; since the total number of pods is B = Anb, the number of pods with mutations will be

$$B_m = B - B_0 = Anb(1 - e^{-M}),$$

whence it follows that

$$m_b = \frac{B_m}{B} = 1 - e^{-M} \qquad (9)$$



or

$$M = -\ln(1 - m_b). \qquad (10)$$

Formula (10) was also proposed by A. I. Mueller (1965).

Finally, let us turn to $m_a$. /85

With an average number of mutations perinitial cell M and a Poissonian distribution of mutations by cells, the probability of the situation that there will not arise mutations in any cell is expressed by the formula:

$$P_0(n) = e^{-M}.$$

Furthermore, it is quite possible to assume that mutant and nonmutant cells are distributed between the embryos of M randomly, and then the probability of a situation where there will not occur any mutation in any of the n initial cells of any embryo will be:

$$P_0(A) = e^{-Mn}$$

and the total number of such embryos in specimen A will be

$$A_0 = Ae^{-Mn},$$

whence it follows that:

$$A_m = A - A_0 = A(1 - e^{-Mn})$$

and

$$m_a = \frac{A_m}{A} = 1 - e^{-Mn} \tag{11}$$

or

$$M = -\frac{\ln(1 - m_a)}{n}. \tag{12}$$

At low frequencies of $m_a$, $m_b$, and $m_c$ (less than 1%), the approximate equalities are fulfilled quite satisfactorily:

$$M = -\frac{\ln(1 - m_c)}{f} = \frac{m_c}{f}; \tag{13}$$

$$M = -\ln(1 - m_b) = m_b; \tag{14}$$

$$M = -\frac{\ln(1 - m_a)}{n} = \frac{m_a}{n}. \tag{15}$$

We will note that the application of formulas (8), (10), and (12)-(15) to the experimental data yields values of M which quite adequately agree with each other; consequently, both the very models that establish the links between the registered frequencies of mutants and the average number of induced mutations and the premises postulated at their basis describe quite well the actual situation.

From the models which have been examined, there ensues one significant conclusion: the independence of estimates of M derived on the basis of $m_b$ and $m_c$ from the number of initial cells demonstrates that for comparative analysis of the appearance of mutations induced at various stages of ontogeny, there can only be used the estimates of M and m on the basis of $m_b$ and $m_c$, but not $m_a$.

And the final matter: in all constructions effected in this chapter, the coupling among the genes was not taken into account. But we can believe that the error introduced by such a simplification would prove to be material only for very large values of M (at any rate greater than one), a situation which we virtually do not encounter in experiments dealing with mutagenesis.

# PART THREE

# SOMATIC AND GENETIC EFFECTS IN ARABIDOPSIS THALIANA (L.) HEYNH. RESULTING FROM SEED IRRADIATION

This part of the book represents a summary of original data /86
on the effect of various dosages and conditions of irradiation of arabidopsis seeds on the survival, growth and fertility of the $M_1$-generation plants and the mutation frequency in the $M_2$-generation. The effect of gamma irradiation on dormant seeds (Ch. 9) served as the basic standard against which the influence of various factors and conditions of the irradiation results were judged in all experiments. During selection of the factors and conditions which modify the result of the radiobiologic reactions under study, preference was given to those whose modifying effect was amply well known from radiobiology of other plants and the effect mechanisms of which are more or less non-specific. These requirements were the natural result of the goal of the experiments--to study the interrelationships among various radiation effects. In this respect, the influence was studied of such modifying factors as pre-radiation wetting of the seeds (Ch. 10), their post-radiation storage (Chp. 11), thermal shocks (Ch. 12), and the radiation LPE (experiments with neutrons, Ch. 13). The sum total of the results obtained in these experiments served as the basis for comparative analysis of the interrelationships among irradiation effects in arabidopsis (Ch. 14).

Each of the chapters in this section of the book is prefaced by general remarks, in which are reviewed data in the literature relevant to this problem; the details necessary for a characterization of experimental methods are briefly described; finally, the results obtained in original experiments are examined and discussed.

## Chapter 9. The Effect of Gamma-Irradiation on Dormant Seeds

### 9.1. General Remarks /87

Seeds of the cruciferae family are among the most radiologically stable among the higher plants (Preobrzhenskaya, Timofeyev,-Resovskiy, 1962; Sparrow, 1965), and the species which interests us occupies one of the first places in this family in terms of radiologic stability. Thus, during comparative study of the stability of the seeds of 47 species of cruciferae to x-rays of 250 kV, it was found that $D_{50}$ for loss of germination capacity (measurements on 15th day) in A. thaliana and in still 8 more species was over 400 curies (the maximum dosage used in the experiments), and for $LD_{50/60}$--240 curies, the same as for Sinapis arvenis L. and Brassica juncea Coss. (Gomez-Campo, Delgado, 1964). The seeds of the remaining 44 species proved to be less stable. In this respect, arabidopsis is a very interesting object for the study of the mechanisms of radiologic stability--especially if we consider the possibility of mass cultivation of this plant under homogeneous conditions, the extensive possibilities for varying the accompanying factors and the ability of use even relatively weak-penetrating radiations.

However, in connection with the fact that arabidopsis was first suggested by F. Laibach as an object for genetic studies, the first experiments involving irradiation of arabidopsis seeds were undertaken not with a view to a radiobiologic study of this plant, but for the purpose of obtaining mutations. The first radiation-induced mutations in arabidopsis were obtained by E. Reinholz in 1947 in the X2-generation after irradiating the seeds of the En-1 race with dosages ranging from 750 to 6000 r (Reinholz, 1947, 1947a). It was she who also published the first results of true radiobiologic experiments on arabidopsis in which was the studied the influence of low temperatures (liquid air) on the radiologic sensitivity of arabidopsis seeds as defined by their germination capacity (Reinholz, 1954, 1962), and also the radiomorphoses of cotyledonous leaves during irradiation at various stages of embryogenesis (Reinholz, 1959).

After this other works devoted to special problems of radiobiology and radiogenetics began to appear, such as, for example: the influence of cultivation temperature (20° and 27°C) on plant survivability after massive irradiation of dry seeds --150 curies by gamma rays of $Co^{60}$(Daly, 1960); the influence of the various dosages of gamma and neutron seed irradiation on the variability of the quantitative characteristic "blos- /88
soming date" in subsequent generations (Daly, 1960, 1961); the influence of the duration of seed wetting before irradia-

tion on the frequency of induced chlorophyllic mutations in the X2-generation (Roebbelen, 1963a, 1965); the relative effectiveness of 14 MeV neutrons and fission neutrons with gamma rays of $Co^{60}$ and $Cs^{137}$ in inducing somatic mosaicism (Fujii, 1964), and so forth. Experiments on arabidopsis irradiation were as before most frequently conducted for the purpose of producing and subsequently studying various types of mutations (Langridge, 1955, 1958; Roebbelen, 1957, 1958, 1960, 1962; Redei, 1960, 1962; McKelvie, 1961; Mueller, 1963; Arnold, 1964). Thus until 1964, when the experiments described below were begun, there had been published only one general radiobiologic article in which were presented the results of experiments on the study of the influence of various dosages of arabidopsis seed irradiation on plant survival, growth, development and fertility (Buiatti, Lorenzoni, 1963). However, the results of these experiments could not provide complete enough radiobiologic characteristics of arabidopsis since the radiation dosages of 20, 40 and 80 curies used on dormant seeds proved to be too small; and the authors could only note that some inhibition of plant development was observed only for the largest of the dosages used.

Therefore, in beginning the study of some associated factors on the somatic and genetic effects of arabidopsis seed irradiation, it was necessary to obtain iniital data on the effect of the irradiation on dormant seeds of this plant. The experiments were begun with standard $Co^{60}$ gamma radiation and conducted with attention to the accumulated experience in the radiobiology of the plants, experience which as early as 1946 was generalized by L. P. Breslavets (cf. page 41) in the form of the following conditions necessary for obtaining the radiobiological characteristics of plants:

1) precise dosimetry;

2) derivation of curves loaded with dosage points across a broad spectrum;

3) extensive samples;

4) equality of conditions (temperature, humidity) both during as well as after irradiation;

5) thorough and comprehensive phenologic observations;

6) growth measurements;

7) record keeping and analysis of yield.

At that time, Breslavets drew attention to the fact that the

data summarized in her book demonstrated a dependence of radiobiologic effects in plants on irradiation conditions and cultivation and that a great deal of attention had been paid to this in the studies of various authors. However, a comprehensive description of the characteristics of irradiated plants is provided much more rarely. And this, of course, is to be much regretted since a lack of knowledge about an object of research undoubtedly reduces the opportunities for analyzing the results of any specialized experiments on such an object.

The results of two series of experiments devoted to the /89
study of the effect of gamma radiation on dormant arabidopsis seeds form the basis of this chapter as well as the data of those variations of other experiments in which dormant seeds were subjected to gamma radiation (in one case X-rays) without any additional effects, and they were sowed immediately after irradiation.

These data are presented here in summary form for the first time, and the results of individual series of experiments are cited in the works of the author and his colleagues (Ginter, Ivanov, 1971; Ivanov, 1967, 1969, 1970, 1971; Ivanov, Ginter, Glotov, 1970; Ivanov, Zyablitskaya et al., 1969; Ivanov, Ivanova, et al., 1967; Ivanov, Sanina, 1967; Ivanov, Sanina, Timofeyeva-Resovskaya, 1967; 1968, 1968a, 1969, 1969a, b; Nikolov, 1968; Nikolov, Ivanov, 1968, 1969; Sanina, 1960; Sanina et al., 1970; Timofeyev-Res vskiy et al., 1971).

## 9.2. Methods of Experimentation

General information on methods used in radiobiologi studies of plants have been presented in Chapter 6. Here we will limit ourselves to the concrete data relevant to the experiments in question.

The basic methodologic information on all the series of experiments whose results are given in this chapter is shown in Table 7.

Common to all the experiment series listed in Table 7 is the fact that air-dried seeds of the En-1 race (water content about 5%) 2-4 months after yield (in order to produce in this race maximum germination capacity) always served as the test material. In all cases the test plants were grown in test tubes on an agarized mineral medium under the following conditions: light period--20 hours, dark--4 hours, temperature about 25°C during the light period and 19°C during the dark period, air humidity 70-80%. The techniques employed in mass laboratory cultivation of arabidopsis are described more fully in Chapter 4. For the M1-generation were recorded the survival

TABLE 7. BASIC DATA ON TESTS WHICH PRODUCED INFORMATION ON THE EFFECT OF GAMMA-RADIATION ON DORMANT ARABIDOPSIS SEEDS

| Test | Content of Test | Radiation | Dose in krads per hour | Gamma radiation krad | Volume of material M1 (# seeds/version x # versions x # tests) | Total volume of material M2 (roughly) |
|---|---|---|---|---|---|---|
| 1 | effect of gamma rays on dormant seed | gamma rays $Co^{60}$ | 220 | 0,10, 20,40,70 110,160, 220,290 | 40x9x5=1800 | 38,000 |
| 2 | same | same | 220 | 0,10,20, 40,70,110 | 60x6x3=1080 | |
| 3 | effect of gamma rays on dormant, swelling and swollen seeds | " | 210 | 0,2.5, 5, 10,20,40 | 20x18x10=3600 | 175,00 |
| 4 | effect of post-radiation storage of seeds on effects of gamma radiation | " | 200 | 0,10,20, 40, 80 | 40x9x5=1800 | 135,000 |
| 5 | " | " | 200 | 0,50,100, 150,200 | 40x9x3=1080 | --- |
| 6 | " | " | 200 | 0,20,40, 60,80 | 40x9x6=2160 | 170,000 |
| 7 | " | " | 200 | 0,50,100, 150,200 | 40x9x5=1800 | -- |
| 8 | effect of thermal shocks on effects of gamma radiation of seeds | $Cs^{137}$ gammarays | 42 | 0,40,80 | (120+30+60) x20= 4200 | 77,000 |
| 9 | comparison of effectiveness of X-rays and neutrons of 5.6 MeV | X-rays 245 kV | 6 | 0,10,20 30,40 | 40x9x6=2160 | 95,000 |
| 10 | comparison of effectiveness of gamma rays and neutrons of 2 MeV | Gamma rays $Co^{60}$ | 14 | 0,25,50, 75,100 | 50x4x4+60x6x x4=2240 | 105,000 |

of the plants in subsequent phases of development by phases and plant fertility. Methods used to record these characteristics are described in Chapter 6. The frequency of induced mutations was recorded for M2: only chlorophyllic mutations among M2 shoots (series 1, 2 and 3), or chlorophyllic and morphologic mutations among M2 plants (series8), or, finally, embryonic and chlorophyllic mutations among M2 embryos in unripe M1 pods (series 4, 6, 9 and 10). Chapters 7 and 8 are devoted to methods for the identification and quantitative record-keeping of induced mutations.

As already noted above, all the experiments were planned using a pattern of randomized sets, and their results were processed statistically using methods of dispersion analysis.

First somatic effects observed in arabidopsis after irradiation of dormant seeds and then induced mutations are treated in the following two sections of this chapter.

## 9.3. Somatic effects

Summary data on somatic effects in arabidopsis observed after gamma-irradiation of dormant seeds are given in diagrams 13-22, where the values of the characteristics are given in percentages deviating from an unirradiated control group along with indicated error limits. In these diagrams the results of indi- /91
vidual series of experiments as numbered in Table 7 are shown by fine broken lines, while the smooth heavy lines coreespond to averaged data. In order not to overload the initial sections of the curves with experimental points, on some diagrams are presented the results of not all the series of experiments in which the corresponding characteristics were recorded, but only the data of experiments involving seed irradiation in which large dosages were used.

An overall comparison of the data obtained indicates that all the recorded somatic characteristics (except seed germination capacity and the duration of the blossoming-maturation phase) distinctly and positively changed with an increase in the radiation dosage and that the relative changes in the characteristics shown in the diagram varied in a number of cases among the experiment series within fairly broad limits.

One more remark of a general nature: as already noted, the data presented in Table 2 (Ch. 5) on the average values and limits of variability of the characteristics in question in the control group convincingly indicate that unirradiated seeds were characterized by very high germination capacity, and that the plants produced from them almost all survived, grew vigorously, developed and bore abundant fruit.

Let us consider now in sequence the results obtained.

### Plant Survival

In Figs. 13-15 are presented the dosage-effect curves for seed germination capacity, plant survival in successive phases of development (cotyledons, rosette, generative phase), and also for the overall survivability of the plants from shoot formation to fruiting.

In Fig. 13 it can be seen that within the dosage range used

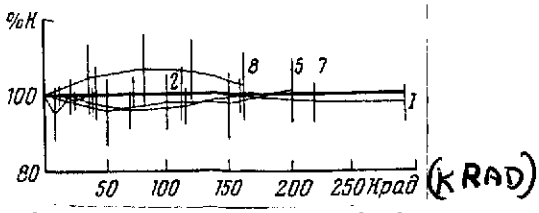


Fig. 13. Ascendability of seeds at different dosages of gamma radiation in dormant state: Figures--numbers of test series in Table 7.

(practically speaking, up to 300 krad) germination capacity of arabidopsis seeds in test-tube culture (very high in the unirradiated control group) is not reduced under the influence of gamma-irradiation in a dormant state. This observation agrees well with the data of other authors (Reinholz, 1954, 1962; Roebbelen, 1962a; Gomez-Campo, Delgado, 1964; Fujii, 1965). Special experiments have shown that a substantial reduction in seed germination capacity in arabidopsis on an agar medium (which corresponds to the so-called laboratory germination capacity) is observed only for gamma-irradiation dosages on the order of 500-1000 krad in contrast to their germination capacity in a soil /92 culture (field germination capacity), when a noticeable influence of gamma- or X-ray irradiation on seed germination capacity may be detected for dosages on the order of 15-20 krad (Kasyanenko, 1964a). The reason for such a great difference is obvious: seeds planted in the soil need not only to germinate but also to penetrate to the sun-lit surface.

It is true that under conditions of a test tube culture for dosages on the order of 100 krad and more, the kinetics of germination in irradiated seeds may lag noticeably behind the control group (Devi et al., 1964; Kucera, 1966), but not to such an extent as to substantially affect the rate of subsequent plant growth.

The exceptionally high stability of germination capacity in arabidopsis seeds (as well as in many other plants) against irradiation is most likely associated with the fact that arabidopsis seeds are characterized by a completed type of embryogenesis; consequently, the swelling of the embryo, the elonga- /93 tion of the hypocotyl and rootlet and the inversion of the cotyledons which constitute the germination process are not associated with cellular fissions but are in essence to a great extent a colloidal-chemical rather than a biologic process. Therefore, a comparison of the retardation of germination capacity under conditions of irradiation along with other radiobiologic reactions occurring in arabidopsis is of no interest. All the more since when additional effects beyond those of gamma-irradiation (post-radiation seed storage, thermal shocks) as well as gamma irradiation of swelled seeds are used, the germination capacity of such seeds in test tube culture also did not change.

In Figs. 14 and 15 are presented the dosage-effect curves for plant survival in subsequent phases of development and also for overall survivability prior to fruiting (Fig. 15b).

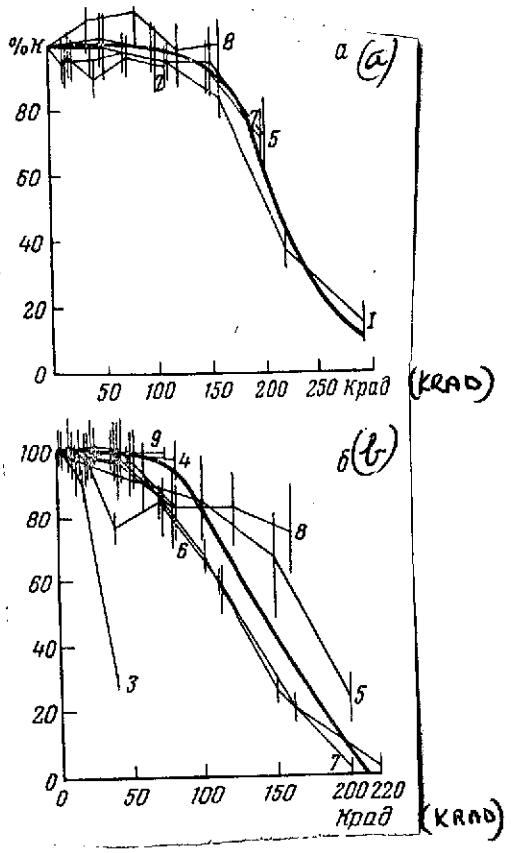



Fig. 14. Survival of shoots in cotyledon (a) and rosette (b) phase at different doses of gamma-irradiation of dormant seeds. Figures--numbers of series in Table 7.

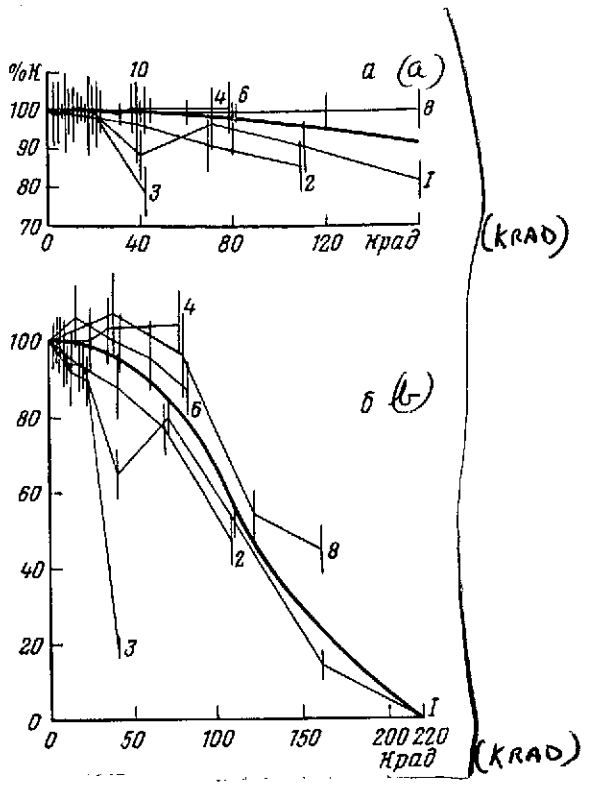



Fig. 15. Survival of plants in generative phase (a) and their total survivability from sprouts to fruit-bearing (b) at different doses of gamma irradiation of dormant seeds. Figures--numbers of series in Table 7.

It can be seen that dormant arabidopsis seeds proved to be highly radiostable since the average $LD_{50}$ value for shoot survivability in the cotyledonous phase was on the order of 200 krad, for plant survival in the rosette phase--on the order of 150 krad and for overall survivability to fruiting--on the order of 120 krad. Here, as for irradiation of seeds of other species, their loss coincided with the earliest stages of their development--cotyledons and rosettes. Plants which had survived these 'critical' phases as a rule achieved fruiting. Although in some series a small percentage of plant losses in the generative phase (about 20% for a dose of 160 krad) was statistically significant (Fig. 15a), such a result was not always repeated for the same radiation doses in other test series. There were in-

stances in which in large test series every plant which had achieved the budding phase survived until the end of the experiment. Thus, the loss of the plants in the generative phase is not a well defined characteristic suitable for assessing the effect of irradiation on arabidopsis seeds.

The early loss of shoots in the cotyledonous phase is apparently due to massive radiation injury in the embryos of the irradiated seeds. This is also attested to by the high doses at which such losses are observed and the great width of the initial plateau of the dosage-effect curve and its subsequent sharp drop (Fig. 14a). Though this may be qualified by stating that the nature of the curve obtained and the degree of 'saturation' do not allow us to subject its shape to rigorous analysis using the methods of quantitative radiobiology. The generally known considerable regenerative capacilities of the plant meristems, capable of recovering even after lethal damage to a considerable portion of their cells (Hall et al., 1963) speak in favor of the massive injury to meristemic cells as the cause of shoot loss. In the case of the early loss of arabidopsis shoots under consideration, no macroscopically establishable growth signs are detected: having opened up, the cotyledons, those shoots destined to perish, completely cease further growth and the development of both the above-ground part as well as /94 the root; there appear no signs of a growth cone between cotyledons; the pigments of cotyledonous leaves fade and the shoots dry up and die. One may assume that the embryonic meristem in such shoots is either completely destroyed or the number of viable meristemic cells is so small that regeneration of the meristem becomes impossible.

The loss of plants in the rosette phase may be assigned a completely different set of radiobiological characteristics. Firstly, this form of loss becomes perceptible and reaches 50% of the number of shoots which have successfully weathered the cotyledon phase, when 'cotyledon loss' is just being felt. Secondly, the dose-effect curve for plant survival in the rosette phase does not have such a wide plateau, and the percentage of surviving plants decreases fairly evenly with dosage increase, i.e., the dose-effect curve in this case has only a small shock effect. By shock, as applied to whole multicellular formations, which are what the embryos of plant seeds are, we should understand, of course, not the primary local occurrences associated with energy absorption in intracellular structures, but the loss, inactivation or any other damage to the cells. It is true that in this case it is more difficult to speak of the shape of the dose-effect curve: in Fig. 14b it can be seen that in relation to 'rosette loss', individual test series differed greatly from one another. Thirdly, 'rosette loss' is a very unusual kind of loss, and some degree of arbitrariness is found in its definition as 'loss.' The problem is that the plants assigned

to the category of those lost during the rosette phase do not die immediately but continue over a long period of time measured in months for form more and more rosette leaves (a total of up to 30-40 versus a norm of 5-6). These leaves are often of abnormal size and shape: small, almost sessile or hair-shaped. The root system continues to grow and branch for a long time. Finally, such plants nonetheless die without having begun generative development. However, vegetative development which continues for a long time without doubt proves that the meristemic tissues in these plants are alive and continue to function, but there exists some kind of obstacle which makes the transition to generative development impossible for them. And, finally, fourthly, of some interest for an understanding of the mechanisms of 'rosette loss' is the fact that in the embryo of a dormant arabidopsis seed the future generative tissue is represented by a very small number of initial cells (1-5) which, as was indicated in Chapter 8, is confirmed by the results of genetic analysis of the structure of the radiation chimeras. If we combine everything stated above, i.e., the small number /95 of initial cells of generative tissue in the embryonic meristem of dormant arabidopsis seeds, the relatively small doses (for arabidopsis) for which is observed the 'rosette type of loss', the absence of a broad plateau in the corresponding dose-effect curves and their relatively gentle slope, and also add to all this the anatomic data on the coincidence of initial cells within the reproductive meristem with very definite histologic structures--the second layer of tunic (Vaughan, 1952, 1955; Vaughan, Jones, 1953)--then we may draw the conclusion that the 'rosette type of loss' (or, as it may also be characterized, the impossibility of a transition to generative development) for irradiation of arabidopsis seeds results from the loss or inactivation of very definite cells of the seeds' embryonic meristem. And this means in turn that within a whole embryo there must exist a certain specialization of cells, and correspondingly, the impossibility of their complete interchangeability. A conclusion, generally speaking, not trivial when speaking of plants.

At first glance, this conclusion sharply disagrees with the entire centuries-old practice of vegetative reproduction of plants by grafts, leaves and even pieces of plant tissue and all the more, with experiments on the regeneration of whole plants from individual cells. But this is only at first glance. In actuality, during vegetative reproduction of plants or during their regeneration from individual cells there takes place an artificial removal of some part of the whole plant from its native surroundings, and thus also the interruption of all cellular interactions, the removed part develops independence and is placed in completely different conditions which cannot have an indifferent effect on the ability of cells to

manifest this or that potential. After all, it is known that the nuclei of animal cells removed from differentiated tissues, the specialization and oligo- and even unipotential of which in situ is taken for granted, are capable under certain conditions of providing the beginnings of a new full-fledged organism as a result of having been transplanted into enucleated zygotes.

One may also object that some 'physiological imbalance' forms the basis of the impossibility for arabidopsis plants grown from irradiated seeds to start generative development--an imbalance such as one which is observed without irradiation when plants are grown under temperature conditions or a light/dark period ratio which does not correspond to their genotype. In such cases, the entire cellular material necessary for the transition to generative development is present; however, this transition does not occur at all or is severely delayed since the conditions needed are absent either for the production of 'florigen' by the plants or for the destruction of substances inhibiting the transition to the generative phase. However, such a physiologic explanation seems less probable to us. Against it also speaks the fact that the impossibility of the transition to generative development manifests itself as an extreme radiobiologic reaction occurring in a portion of the irradiated total number of plants, evenly increasing with dosage, whereas the remaining plants continue to develop more or less normally, blossom and bear fruit, and this indicates besides that the conditions of development, optimal for the control plants, are favorable for /96 them as well. The assumption that stable changes in the normal type of reaction to conditions of medium (given the interchangeability of meristemic cells) appear in a part of the plants under the influence of seed irradiation is too improbable to warrant taking into consideration.

The effect of physiologic mechanisms assumes a more or less even, or in any event more or less normally distributed injury to the entire amount of irradiated plants. Besides, as dosage increases it is not the number of extremely injured individuals, but the extent of injury of the whole group of plants should increase, i.e., a cumulative effect should be observed. On this basis we may assume that the effect of such physiologic mechanisms resulting from radiation injury more likely forms the basis (if only partly) of such radiobiologic reactions in arabidopsis as retardation of plant growth and development in individual phases, inasmuch as it is precisely these reactions which are manifested quantitatively as well as changes resulting from varied dosage of the average values of characteristics which vary moderately within the limits of the whole group of plants under study. These reactions will be discussed below.

It may be assumed also that the late loss of plants observed in the generative phase of their development is, if only partially, the result of the effect of physiologic mechanisms.

In this case, the transition from vegetative to generative development is not ruled out, but, having formed a weak floriferous shoot usually with a defective and considerably reduced raceme bearing only rudimentary embryos of blossoms, these plants are not in a position to complete development and they die, their loss coinciding with any particular period of development, but stretching out in time from the start of the budding phase to the end of the blossoming phase. Such a picture allows us to think that in this case, the material minimally necessary for differentiation of tissue had been preserved, the reproductive organs being formed are poorly supplied with nutrients from the vegetative organs which are weakly developed as a result of the irradiation. This point of view is all the more justified by the fact that in the generative phase only plants having extremely poorly developed rosettes leaves and root system die. However, even in the lat loss of plants the effect of other causes besides their general dystrophy is possible. This applies especially to those instances when the plants form racemes but with degenerative or abnormally developed blossoms and only later die without fruiting, since the reproductive parts of the blossoms are either lacking or abnormal. Such a form of late /97
loss associated with radiomorphoses of the blossoms may be the result of dominant mutations of genes which control blossom development, mutations occurring in the initial cells of generative tissue. Of course, the term dominant mutations is used here in the broadest possible sense and means only that such changes appear in the diploid generation subjected to irradiation; the genetic nature of such changes lacks concrete expression.

In conclusion, let us comment that in mentioning the physiologic mechanisms of radiobiologic reactions under consideration here, we have in mind either general dystrophy of the plants forced to regenerate from a relatively small number of remaining live meristemic cells or stable changes passed on from one generation to the next, since the recorded reactions (except cotyledonous loss), generally speaking, are the remote results of irradiation observed only after the plants produced from irradiated seeds undergo a whole series of growth and development stages. Both these mechanisms most likely are significant, the extent to which they contribute to various reactions being different. Various reversible and non-reproductive pathologic conditions within the cells appearing as a result of irradiation can at best be of only secondary importance.

Thus, the overall lethal effect observed for irradiation of

dormant arabidopsis seeds and quantitatively represented by the dose-effect curve in Fig. 15b results from the loss of shoots in the cotyledonous phase due to massive injury to meristemic cells of the embryos; also from plant loss in the rosette phase due most likely to destruction of initial generative tissue cells, and the late loss of plants, quantitatively poorly reflected, resulting from various causes.

It is natural that for purposes of comparing various somatic and genetic effects of irradiation, the first two forms of plant loss are of particular interest--'cotyledon' and 'rosette' loss as they are more specific and quantitatively well-defined. Late plant loss is of less interest as it is quantitatively less well-defined. The overall lethal effect is by its nature too heterogeneous. Therefore, when comparing the influence of factors associated with irradiation on various somatic and genetic reactions in plants, we will consider only the 'cotyledon' and 'rosette' forms of loss.

Plant Growth

As noted in Ch. 5, plant growth was measured in our experiments using the following parameters: length of main root on the 7th day of the experiment (Fig. 16a), or its length on the 7th day after seed sprouting (Fig. 16b); overall plant height on the 21st day of the experiment (Fig. 17a) or plant height /98 on the day of the start of blossoming (Fig. 17b).

Of course, such characteristics as root length on the 7th day of the experiment and plant height on the 21st day of the experiment have incorporated within them components which described the rate of plant growth, since the measurements were in this case taken for the same calendar periods by which plants of various types achieved various phases of development.

When we make a generaly analysis of the data presented in Figs. 16 and 17, our attention is drawn to two points. First, the growth characteristics exhibit considerable variability. This is particularly true for their reproducibility in various experiment series. The causes of this variability are understandable: it is due to the well-known plasticity of plant growth and the substantial contribution to the overall variability of these characteristics of the medium component in the/99 broad sense of the word. Second, serious inhibition of growth is already observed for those doses where the lethal effect is entirely absent or has only a weak presence. This is especially true of root growth characteristics for which $D_{50}$ is only 40 to 60 krad, the dose-effect curves for both root growth characteristics very much resembling each other in form and the value of isoeffective doses (Fig. 16a, b). In both cases the dose and

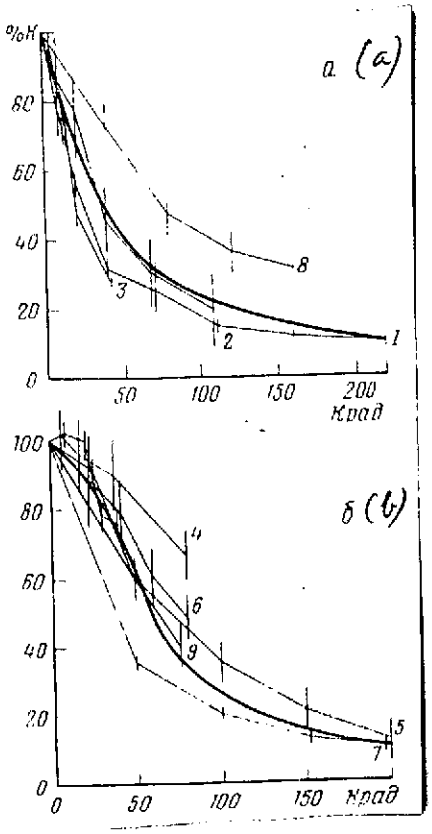



Fig. 16. Length of root on 7th day after sowing (a) or appearance of shoots (b) at different doses of gamma irradiation of dormant seeds (figures are numbers of test in Table 7).

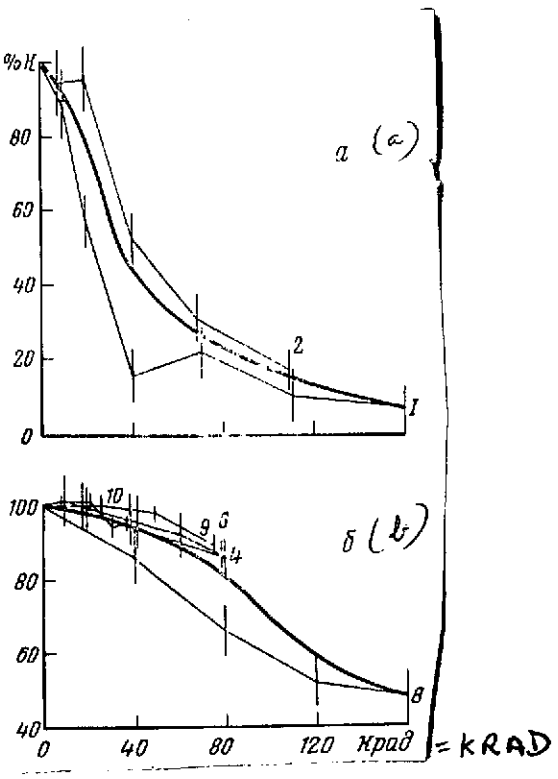



Fig. 17. Height of plants on 21st day of test (a) and on day of start of blossoming (b) at different doses of gamma irradiation of dormant seeds (figures--Tab. 7).

effect curves are characterized by a fairly sharp drop for small doses after which they bend and subsequently slowly sink to some minimal value so that they may be described as being composed of two components. In many test series, the sharp drop in these curves in their initial portions is depicted by a linear or nearly linear dependency on the irradiation dose which is apparently due to the approximate dose-proportionate reduction in the number of living meristemic cells capable of dividing, cells on the basis of which the root system of the plants develops. So far as the second (slow) component is concerned, it describes that 'minimal' or 'residual' root growth of which the plants are capable only if they were alive at the time of measurement.

Finally, let us note that the considerable resemblance in the dose-effect curves for both root growth characteristics confirms, as pointed out above, that the small delay in seed sprout-

ing observed for irradiated seeds little affects the subsequent development of the plants; therefore both these characteristics to an equal extent are suitable for quantitative measurement of root growth and its inhibition for irradiated seeds.

Inhibition of the growth of the above-ground portion of the plants which affected their overall height on the 21st day of the experiment (Fig. 17a) is depicted by a dosage dependency similar to that of root growth inhibition. However, as noted above, this characteristic is a multifaceted one since it reflects not only retardation of plant growth but also a delay in their development, since at the time of measurement the plants subjected to various types of irradiation attain various phases of ontogeny. In this respect, in 'pure form' the growth of above-ground portions of plants and changes in them resulting from seed irradiation can be better described by their height measured at the same biologic time. In a test tube culture (particularly sterile, when plants grow under stoppers) the most convenient measurements of arabidopsis plants can be made the day the first flower blossoms: by this time the plants are already sufficiently high (6-8 cm in the control group) but have not yet reached the stoppers, and thus their stems are not bent. This characteristic of the growth of the above-ground portion of the plants was considered during the experiments, the results of which are presented in subsequent chapters. The dose-effect curve for plant height on the day of initial blossoming for dormant seed irradiation is shown in Fig. 17b. It can be seen /100 that is differs considerably from the corresponding curve for plant height measured on the 21st day of the experiment. First, for dormant seeds exposed to doses of gamma rays up to 50 krad, the plant height on the day of initial blossoming does differ in no way from the control group, and second, the plant height reduces slowly for great doses so that for a dose of 160 krad (maximum dose at which a number of plants still survive through blossoming) vertical growth of the plants still attains 50% of that of the control group. Apparently the plants can proceed to generative development only provided that their trophic resources can ensure at least half of stem growth as compared with that of the control group. In this respect it is difficult to state with sufficient certainty what is the primary cause of inhibition of stem growth for irradiated seeds; injury to the cells of the reproductive meristem as such or overall injury to any cells of the embryonic meristem on account of which the plants are forced to develop from a less than normal number of meristemic cells left uninjured (in any case lethally) which in turn results in a depletion of the trophic resources of the developing plants.

Plant Development

As noted in Ch. 5, the rate of vegetative and generative plant development was taken into account using two different methods: in more approximate terms by the percentage of rosettes which had formed by the 7th day of the experiment and the percentage of racemes which had formed by the 21st day of the experiment, and in more precise terms--by the average duration of subsequent phases of development.

The data obtained using the first method in three test series are presented in Fig. 18a and 19a, where it can be seen that the percentage of rosettes formed by the 7th day of the experiment and the percentage of racemes formed by the 21st day drop sharply with an increase in gamma irradiation of dormant seeds, being characterized by considerable variability. The sharp deviation of the data of the third series of experiments from average values also draws attention to itself. Owing to such variability as well as in the case of the lethal effect of the radiation, the shape of the dose-effect curves for the rate of plant development scarcely deserves detailed discussion. In general terms with consideration given to the data of individual experiment series, the dose-effect curve for vegetative development (Fig. 18a) may be described as a more or less even dose-proportionate decrease in the percentage of rosettes (of their total number) which had appeared by the recorded (7th) day of plant development. This nature of dose dependency is most likely due to the fact that rosette leaves in arabidopsis /101 are formed from a large number of cells within the vegetative part of the upper embryonic meristem. This is proven by both the above quoted anatomic studies (Vaughan, 1952, 1955; Vaughan, Jones, 1953) and the rare occurrence and small size of mosaic spots on the first pair of rosette leaves for irradiated seeds of heterozygotic lines (Fujii, 1954). As seed irradiation dose increases, the number of living cells within the vegetative meristem capable of fission more or less evenly decreases; accordingly, a more or less even decrease in the rate of vegetative development in the plants is observed.

The dose-effect curve for the rate of generative development, i.e., in this case for the percentage of racemes (of their /102 total number) which had appeared by the 21st day of plant development, has a more or less well-defined S shape (Fig. 19a). When interpreting this curve, we should remember that during seed irradiation, both the reproduction and the vegetative part of the embryonic meristem are injured. Therefore, we could think that the shape of the curve reflects not only the fate and participation of plants with initial generative tissue cells in the development process, but also the overall readiness of the plants to proceed to the generative phase for seeds exposed

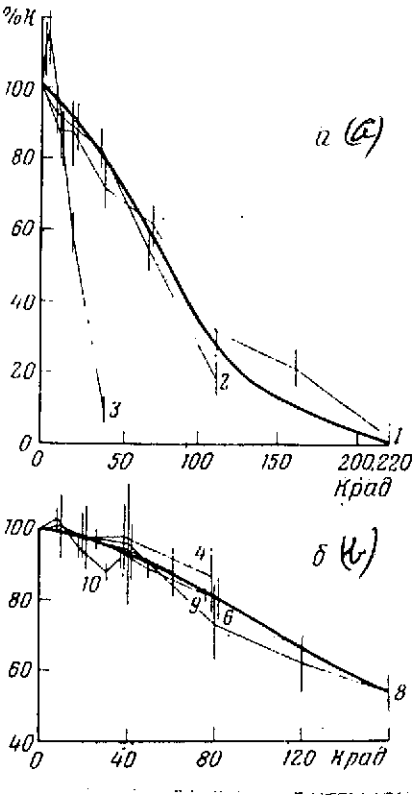



Fig. 18. Rate of vegetative development of plants at different doses of gamma radiation of dormant seeds evaluated by the portion of rosettes formed in the first week of the test (a) and by duration of the phase of shoots-rosettes (b).

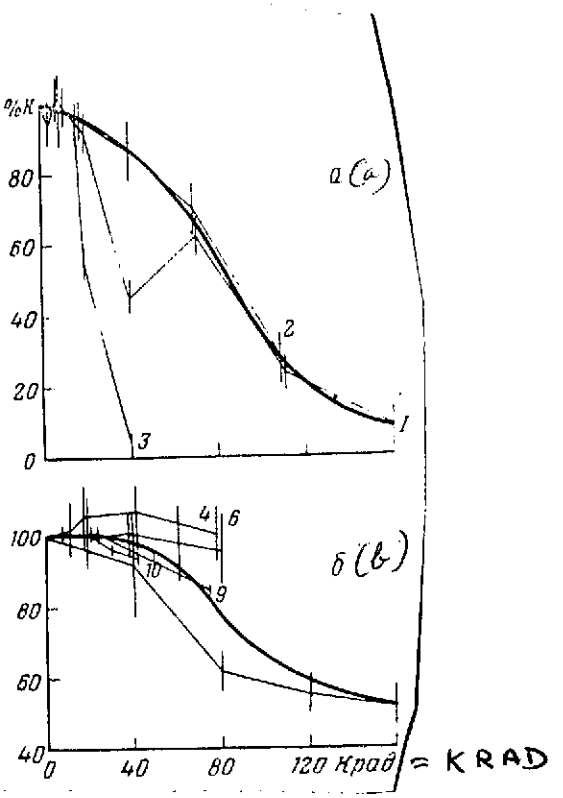



Fig. 19. Rate of generative development of plants at different doses of gamma radiation of dormant seeds evaluated by the portion of racemes formed by the 21st day of the test (a) and by the length of the rosette budding phase (b). Figures relate to test series in Table 7.

to various doses of radiation. Such readiness depends, as it is known, not only on the condition of the reproductive meristem, but also on overall plant growth, the building-up of above-ground and root phytomasses and the development of photosynthetic machinery sufficient to provide the developing generative organs with the necessary 'building materials.' In connection with what has been said, we may think that the gentle beginning part of the dose-effect curve for the rate of generative development in arabidopsis reflects the presence in the upper meristem of some kind of reserve seeds such that for doses on the order of 25-45 krad, when plant growth i half neutralized,

only about 10% of the irradiated plants lag behind in their development when compared to plants of the control group. The subsequent sharper drop in the dose-effect curve for the rate of generative development is apparently associated with the depletion of 'growth reserves' in connection with which the growth and development of the plants takes place only through the use of the 'basic meristemic material', the number of living cells in which progressively decreases with increases in seed irradiation doses. It is difficult to say anything definite about the causes which give rise to the gentle slope of the final section of the dose-effect curve under consideration. However, by way of discussion we can suggest that the nature of this section of the curve is associated with two processes proceeding simultaneously. On the one hand, there is the development of the plants' vegetative organs, considerably inhibited as a result of massive seed irradiation. On the other hand, a reproductive meristem develops from initial generative tissue cells, the rate of its development being determined by the normal type of reaction of the genotype to environmental conditions. In sum, by the end of a certain period the development of a reproductive meristem proceeds so far that, despite trophic insufficiency, the plants nonetheless make an attempt to proceed to the generative phase of development. Not always is this attempt successful, which can be seen from the late loss of irradiated plants observed specifically in this dosage range (Fig. 15a). But, although it seems adequately scientific, we repeat that this is merely a suggestion.

In Figs. 18b and 19b, we can see that the rate of vegetative and generative plant development determined using the average duration of the seedling-rosette and rosette-budding phases is /103 depicted by the dose-effect curves resembling the curves in Figs. 18a and 19a, respectively, i.e., vegetative development more or less evenly slows down with increases in seed irradiation doses, and the change in the rate of generative development is shown by an S-shaped curve. What draws our attention is the fact that for the same irradiation dosages there is considerably less deviation from the control group in the average durations of the development phases under consideration than the corresponding deviations in the percentage of rosettes formed by the 7th day and the percentage of racemes formed by the 21st day. These differences in the size of deviations are associated with the arbitrariness characterizing the selection of time points (7th and 21st days), when the overwhelming majority of control group plants had already proceeded to the next phase of development. Thus, the measurement of the rate of development which uses average duration, free from such arbitrariness, seems more advisable and as better reflecting the actual situation. These are precisely the characteristics which were taken into account in most of the experimental series.

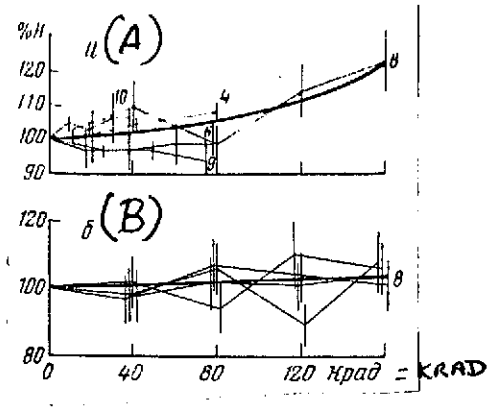



Fig. 20. Rate of development of plants in budding-blossoming (A) and blossoming-maturation (B) phases at different doses of gamma irradiation of dormant seeds. Numbers refer to Table 7.

The developmental characteristics just reviewed taken together reflect the first part of development--up to budding. But radiobiology is only interested in this part. As can be seen from Fig. 20, the length of the later developmental phases of budding and blossoming and blossoming and maturation for all practical purposes is not dependent on seed irradiation. A small acceleration correlatively associated with plant growth may be noted for the first of them: the lower the height of the plants, the faster the budding-blossoming phase proceeds. The duration of the blossoming and maturation phase is in no way dependent on seed irradiation.

We shall make one more remark in summing up our discussion of problems of the lethal effect of gamma-irradiation of seeds in arabidopsis on growth and development inhibition /104 resulting from this irradiation. When discussing in this as well as previous sections possible cellular bases for these or those final manifestations of radiation injury in plants and when speaking repeatedly in this respect of cell loss, not once did we touch on the problem of how and why do the cells die as a result of irradiation. We did this intentionally, since we would have to refer to subsequent chapters in this book to discussion this question. Therefore, the problem of the effect of irradiation on cells is deferred until Ch. 14. Here we may note in the most general terms that of significance for the above considered radiobiologic reactions are those types of injuries to meristemic cells which are incompatible with their full-fledged mitotic activity constituting the basis of plant growth and developmental processes. This distinguishes the above discussed radiobiologic reactions in cells from induced sterility irradiation and also from mutations observed in the following generation. We shall now proceed to a discussion of the latter two problems.

## Plant Fertility

A discussion of the problem of plant fertility and changes in it resulting from seed irradiation in a section devoted to somatic effects is justified by the fact that plant fertility was taken into account in the same M1-generation of plants, whose initial stage (seeds) was subjected to irradiation. And

21 22

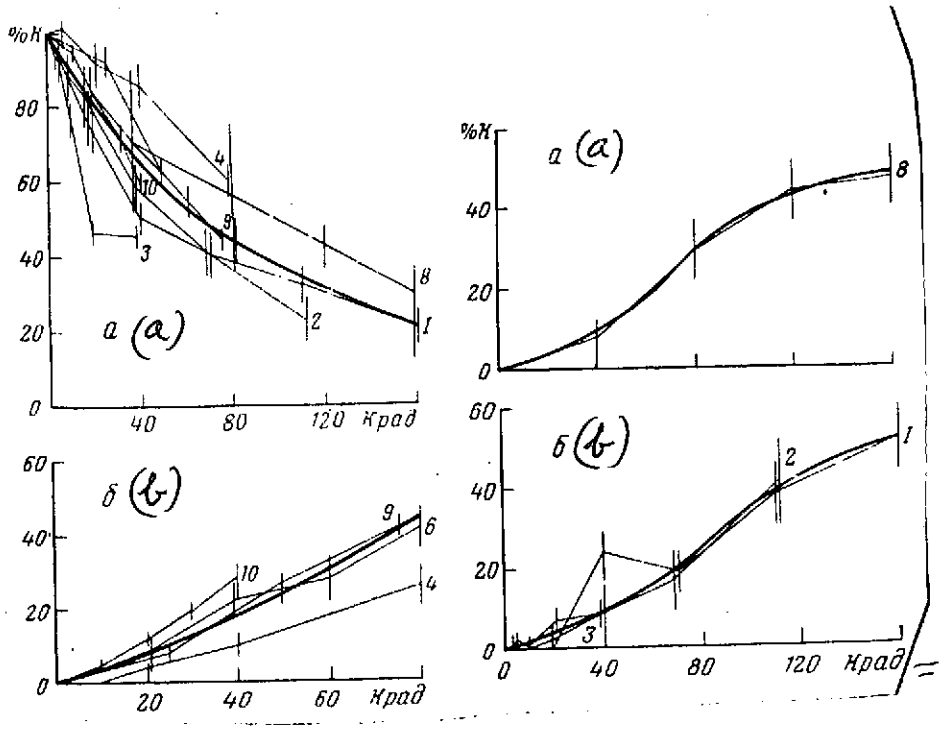



Fig. 21. Number of seeds in pods (a) and frequency of sterile seedbuds (b) at different doses of gamma irradiation of dormant seeds.

Fig. 22. Frequency of sterile polds (a) and sterile plants (b) at different doses of gamma irradiation of dormant seeds.

Numbers refer to test series in Table 7.

in this sense, plant fertility is a somatic characteristic. In addition to this, there are convincing grounds for considering that a decrease in plant fertility under conditions of irradiation, like induced sterility, is the result of a specific class of chromosomal anomalies, freely passing through a number of mitoses, but interfering with the normal processes of meiosis, haplophase and fertilization, whereas the contribution of 'physiologic' sterlity is small (Gustafsson, 1940; Ehrenberg, Lundquist, 1957; Mueller, 1967). And in this sense, the decrease in plant fertility resulting from seed irradiation is a genetic effect. Having postponed a discussion of this problem until Ch. 14, let us now turn to the results of the experiments under consideration here.

In all experiment series plant fertility was measured using the average number of seeds per pod (Fig. 21a). As noted in Ch. 5, defining plant fertility using the average number of seeds per pod, and not the total number of seeds from one plant was determined by purely methodologic considerations--the first of these two characteristics varies considerably less from

experiment to experiment, i.e., it yields better reproducibility of results. So far as induced sterility is concerned, in various experiment series it was measured approximately by the frequency of completely sterile plants (Fig. 21b) or, somewhat more precisely, by the frequency of sterile pods (Fig. 22a) or, finally, by the number of ovules which had not begun to develop (Fig. 22b). The curves presented in Figs. 21 and 22 show that plant fertility exhibits a very subtle reaction to irradiation, varying moderately (versus growth and development). /105 The absence of a distinctly defined threshold is most typical of the dose-effect curve which depicts a change in the number of seeds (Fig. 21a): with an increase in dosage this curve sinks evenly, and for doses of 65-70 krad, the number of seeds decreases to half in relation to the control group. Although the absence of a treshold is a necessary, but as yet insufficient characteristic to consider a reduction in the number of plant seeds not a multifaceted physiologic reaction of a multicellular organism, but a more or less direct result of primary local events taking place at the intracellular level, nonetheless, in combination with other facts (which will be discussed in Ch. 14) the nature of the dose-effect curve may be seen as one of the indirect conclusions in favor of this interpretation of induced sterility. Finally, the dose-effect curve under consideration is characterized by the fact that even for the largest dosage (160 krad) at which a portion of the plants still began the fruiting phase, the number of seeds did not equal zero. This means that within the range of large gamma radiation dosages during seed irradiation, the increase with dosage of the lethal effect in arabidopsis exceeds the decrease in the number /106 of seeds in surviving plants. The same can be seen in Figs. 21b and 22 where the dose-effect curves for induced sterility are presented: for the mmme maximum dosage of 160 krad, all three characteristics by which sterility was measured attained values of 45-50%. A comparison of the shape of the dose-effect curves for induced sterility characterized by various features is scarcely advisable--all of them give only an approximate reflection of the real situation. One may only note their similarities: all of them have a more or less even increase of the effect with dosage increase and, like the dose-effect curve for the seed quantity, do not have a clearly defined threshold.

Thus, somatic effects observed for seed irradiation in arabidopsis are numerous and varied. In radiobiologic terms, they differ both in the dosage ranges at which they manifest themselves as well as in the nature of the dose-effect curves. Using the terminology of classical quantitative radiobiology, they may be divided into weak and strong, single component and non-single component effects. As applied to multicellular or-

ganisms, which in this case is what the irradiated objects--seed embryos--are, differences in the nature of dose curves most likely reflect how many and specifically which cells need to be injured in order to bring about the observed effect. It is not possible to assess only on the basis of the above discussed dose-effect curves the extent to which the cell injuries which form the basis of certain somatic effects in plants resemble or differ from one another. It is necessary to see as a minimum whether factors associated with irradiation will affect somatic effects equally or not. This will be discussed in subsequent chapters of the book. Now let us turn to mutations, the genetic effects of irradiation as such.

## 9.4. Genetic Effects

As noted above, from a large number of genetic effects which were recorded as a result of mutagenic effects on seeds, we obtained dose-effect curves for chlorophyllic mutations recorded among M2 shoots and the sums of embryonic lethals and chlorophyllic mutations recorded among M2 embryos in unripe M1 pods.

Chlorophyllic mutations or, more precisely, leaf coloration mutations (since this group includes also mutations involving other pigments) are the mutation class most loved by genetic botanists (they may be recorded fairly easily and with objectivity, they are not too rare and control one of the most important plant functions--photosynthesis). /107 They were the first radiation-induced plant mutations obtained by L. J. Stadler in experiments on corn (Stadler, 1928), an achievement which represented the beginnings of radiation genetics in higher plants. Stadler was the first to doubt that not only chlorophyl but all radiation-induced mutations produced in higher plants could be considered gene mutations (Stalder, 1932). He subsequently developed this viewpoint (Stalder, Roman, 1948; Stadler, 1954) and at the present time it is shared by many geneticists (Nilan, 1967). This to a still greater extent applies to embryonic lethals. These brief remarks are needed in order to explain just what are the defects which will be discussed below. The problem of the nature of induced mutations in plants is provided a detailed analysis in Ch. 14.

Dose-effect curves for the number of mutations (per 100 cells) induced in arabidopsis as a result of irradiation of dormant seed are presented in Fig. 23a--for chlorophyllic mutations; and 23b--for their sum along with embryonic lethals. A general analysis of the curves shows that for comparable irradiation dosages the number of chlorophyllic mutations is about one order less than the number of embryonic lethals. It can also be seen that the number of mutations (as compared to the somatic

effects of irradiation) is distinguished by moderate variability. A considerable difference was noted only between the number of chlorophyllic mutations in the first and third series of experiments.

As in the case of somatic effects, there is scarcely any point to a detailed discussion of the shape of the dose curves shown in Fig. 23. One may note that they are characterized by the absence of a well-defined threshold, an increase in the number of induced mutations being roughly proportional to the dosage.

In this chapter, which begins the radiobiologic portion of the book, something else is of greater importance: the need to verify, using experimental data, the adequacy of the methods for quantitative analysis of mutagenesis presented in Ch. 8. As already noted, data on chlorophyllic mutations are more appropriate for this purpose, since the very high frequency of embryonic lethals obscures some numerical relationships relevant to the structure of chimeric plants. Methods and formulas from Ch. 8 were used not only for constructing the dose curves shown in Fig. 23, but also for determining the volumes of samples needed for effective record-keeping associated with the developing mutations. Therefore, it was advisable to also verify to what extent the actual effectiveness of record-keeping for the developing mutations deviated from that expected. And for this purpose, data on chlorophyllic mutations obtained from the first two series of experiments involving dormant seed irradiation are more suitable.

The overall pattern of chlorophyllic mutations consisted of three successive stages: 1) the seeding of sufficient number /108 of M1 seeds for detecting mutations which had appeared among M2 shoots; 2) cultivation until fruiting and the production of seeds from a sufficiently large number of phenotypically normal M2 plants in order to detect among them (using M3 data) heterozygotic plants resulting from the mutations; 3) the seeding of a sufficient number of seeds from M2 plants to detect fission in M3 progeny resulting from heterozygotic plants.

The use of heterozygotic plants to confirm the occurrence of mutations is due to the fact that a substantial portion of them are recessive-lethal mutations which result in the loss of the shoots during the cotyledonous leaf phase.

The following served as the basis for assessing the effectiveness of the method and calculation of the necessary numbers of samples. First of all, arabidopsis seed embryos an average of two initial cells of future generative tissue. Given the minimum number of mutations per embryo (one mutation in one of the two homologous chromosomes of one of these two cells)

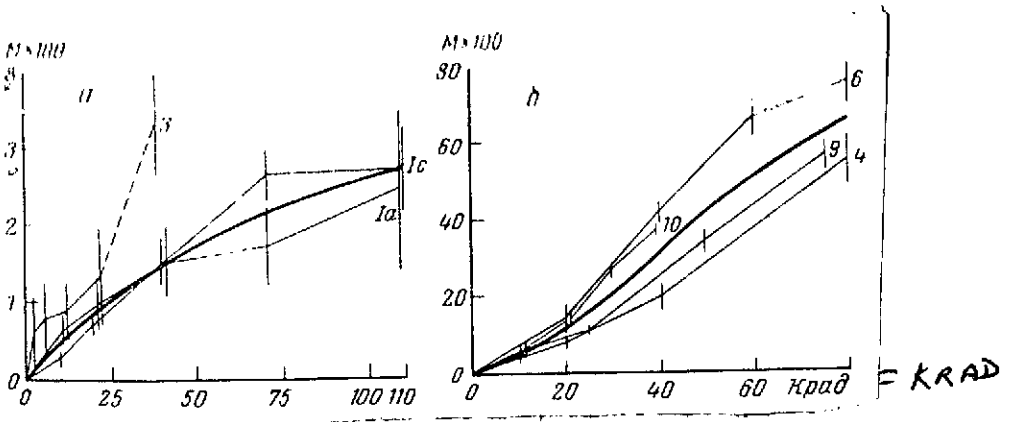



Fig. 23. Average number of mutations per 100 cells at different doses of gamma irradiation of dormant seeds in recording of:
a) chlorophyllic mutations among shoots of M2 (estimation M Ia in terms of $m_a$; Ic in terms of $m_c$); b) sum of chlorophyllic mutations and embryonic lethals in pods of M1. Figures refer to Table 7.

the probability of homozygotization under conditions of self pollination equals 1/8. Secondly, the ratio of mutant and non-mutant sectors in chimeric M1 plants on the average does not change throughout the course of the plants' development, i.e., the mutations occurring are completely recessive and not subject to the effects of ontogenic selection. Thirdly, the location of successive pods in the fruit system is such that their localization on one generatrix is repeated each five pods. And finally, the majority of chlorophyllic mutations are inherited as monogenic differences. Then, should we desire to establish overall effectiveness equal to 0.9 for all occurring mutations, and correspondingly the effectiveness of each of the three stages, equal to $\sqrt[3]{0.9} = 0.967$, we may on the basis of binominal distribution calculate the necessary size of extracts for each of the stages in order that the probability of producing at these stages at least one interesting change equal the accepted level./109 The results of such a calculation yielded the necessary sample sizes: 25 for M2 shoots and 12 for both M2 plants brought to fruiting and M3 shoots. This calculation, in keeping with the adopted premises, is based on probabilities of 1/8 for homozygotization of mutations occurring in M1; 2/7 for the presence of heterozygotic plants resulting from mutations among phenotypically normal M2 plants; and 1/4 for mutations splitting in M3 families produced from heterozygotic plants of M2, i.e., the minimum possible number of mutations--one per embryo--was adopted as the basis. Deviations from Mendelian relationships were not taken into account.

In practical terms, the recording of chlorophyllic mutations using the arrangement presented took place as follows. From each M1 plant a random sample was sown onto a Petri dish (with a small reserve for reduced germination) from 32(but no less than 25) seeds from the first live pods (plants which produced less than 25 seeds were eliminated) and grown with the aid of lighting equipment (light from above and not from the side as for test tubes) for 5-7 days (two families fit conveniently in rows of 4 x 8 on one normal size Petri dish). After this, all sprouts were distinguished visually. Only families composed of 25 or more shoots were taken into account (for the remaining families, given the availability of a number of seeds, it was possible to complete sowing). Families not containing even one shoot with changed cotyledonous pigmentation were classified as normal, and out of the families which yielded at least one changed plant, 16 (4 in reserve in case of loss) phenotypically normal shoots and all the changed ones (a preliminary assessment of their viability) were transplanted to test tubes. The M2 transplanted shoots were grown to maturation. All the seeds from each plant were collected individually and seeded (16 seeds from each family--random selection) onto Petri dishes. If, with the appearance of sprouts in at least one of the M3 families which traced its parentage to a common M1 ancestor, there appeared the same change in pigmentation that was recorded in M2, then we concluded that such a mutation appeared in the embryonic meristem of this M1 plant. Otherwise, we concluded that the change recorded in M2 was the result of modification, and the corresponding M1 plant was classified as normal. At all stages of recording, samples less than the required size (25 and 12, respectively) were eliminated if it was impossible to supplement them from the remaining seeds. Only when this rule is observed do the estimates produced retain their objectivity and avoid bias.

The basic data of the first and second series of experiments on recorded frequencies of chlorophyllic mutations ($m_a$ and $m_c$) /110 for various dosages of gamma irradiation of dormant seeds are given in Table 8. In view of the absence of any substantial differences between the series, both in terms of methods used and the results obtained ($P > 0.1$), the data have been combined. The table contains only those mutations detected in the M2 generation which were confirmed by genetic analysis in the M3. A total of 1204 M1 families were analyzed; among them there were detected 26 independently-occurring chlorophyllic mutations represented by 80 mutant shoots from among 37,564 examined. The first two sections of Table 8 indicate that as irradiation dose increases, both the frequency of splitting families (M1 ($m_a$) and the frequency of mutants M2 ($m_c$) increase, $m_a$ being on the average an order higher than $m_c$.

TABLE 8. RECORDED FREQUENCIES OF CHLOROPHYLLIC MUTATIONS IN THE M2 GENERATION AND SPLITTING IN M2 and M3 GENERATIONS AT DIFFERENT DOSES OF GAMMA IRRADIATION OF DORMANT SEEDS (1 and 2 TEST SERIES)

| Parameter | Dosage, krad | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | 70 | 110 | Total |
| Number of splitting families in M1..... | 1 | 5 | 7 | 6 | 5 | 2 | 26 |
| Total number of M1 families..... | 309 | 291 | 281 | 165 | 123 | 35 | 1204 |
| Number of mutant shoots in M2..... | 6 | 11 | 18 | 18 | 21 | 6 | 80 |
| Total number of shoots in M2..... | 9215 | 9286 | 8973 | 5201 | 3804 | 1085 | 37,564 |
| $m_c$, %..... | 0.065 | 0.118 | 0.201 | 0.346 | 0.552 | 0.553 | |
| Splitting in M2 offspring from chimerical plants of M1: | | | | | | | |
| number of mutant shoots..... | 6 | 46 | 79 | 29 | 62 | 7 | 229 |
| total number of shoots..... | 63 | 534 | 635 | 330 | 388 | 70 | 2020 |
| % mutants..... | 9.5 | 8.6 | 12.4 | 8.8 | 16.0 | 10.0 | 11.3 |
| Splitting in M2 offspring from heterozygotic plants in M2: | | | | | | | |
| number of mutant shoots..... | 6 | 167 | 586 | 141 | 263 | 59 | 1222 |
| total number of shoots..... | 35 | 1022 | 3264 | 861 | 1280 | 358 | 6820 |
| % of mutants... | 17.1 | 16.3 | 18.0 | 16.4 | 20.5 | 16.5 | 17.9 |

In order to refine the data on the frequency of mutants in dividing families, the remaining seeds were sown from all M1 plants in the M2 offspring of which chlorophyllic mutations had developed. The results are presented in the same Table 8, where/111 it can be seen that the frequency of mutants in the dividing M1 families varied from dose to dose, but htis variation was not systematic in nature: it is impossible to trace either a tendency towards growth or a tendency towards a decrease in the frequency of mutants associated with an increase in dosage. Therefore, although the differences between doses proved to be significant ($0.005 < P < 0.01$), serious meaning can hardly be attributed to them. The overall frequency of mutants was 11.3% (+: mut = 7.8:1) which does not significantly differ ($0.1 < P < 0.25$) from the 12.5% expected for division at a 7:1 ratio,

if the average number of initial cells equals 2 and the recessive mutation occurs in one of them in a heterozygotic state and is not subject to ontogenic selection in the chimeric M1 plant, and also if the corssing of gametes originating from different initial cells does not occur. It was specifically these conditions which were the primary ones used in constructing the model in Chapter 8.

In the M2 progeny of M1 chimeric plants, the ratio of dominant homozygotes to heterozygotes among phenotypically non-mutant individuals was 240:83, or 5.8:2, which does not significantly differ ($P = 0.25$) from the ratio 5:2 expected for newly formed conditions. In the M3 progeny of heterozygotic M2 plants, the frequency of mutants (last section of Table 8) was 17.9% (+:mut = = 4.6:1) which is substantially lower than the 25% ($P < 0.001$) expected for division at a ratio of 3:1. No differences between mutations obtained for various irradiation dosages in this case were observed ($0.05 < P < 0.1$). The shortage of mutants may be the result of their reduced viability during various phases of development, including their reduced germination capacity. This is proven by the significant correlation between germination capacity and the frequency of mutants in splitting M1 families ($r^s = 0.43$; $0.01 < P < 0.05$). True, no such correlation was found in M2 families ($r^s = 0.15$; $P > 0.05$), but this may be explained by the generally low germination capacity in this case (85% as against 97% in M1 families) as a result of which genotypic differences in germination capacity proved to have been masked by the high paratypic background. This implies that to establish reliable ratios for splitting along lines of chlorophyllic mutations, we must have excellent seed germination capacity; in addition, a method which records chlorophyllic mutations in maturing pods (Mueller, 1963) is more reliable than recording them in shoots.

Using the observed frequencies of mutants in splitting M1 and M2 families and the frequencies of heterozygotes in M1 families, we performed a recalculation of the total effectiveness of mutation recording, a recalculation which indicated that such effectiveness was 84%. This is close to the figure which was used as the basis of the calculations (90%) and indicates conformity with the experimental data associated with the assumptions forming the basis of the model described in Ch. 8.

Finally, the following initial data were adopted on the /112
basis of the results obtained for calculating the number of induced mutations: average number of initial cells $\bar{n} = 2.2 \pm 0.2$ and the average frequency of splitting of mutants in the progeny of heterozygotes, $f = 0.179 \pm 0.005$.

After this, using the values of $m_a$ obtained (by formula (12))

and the values of $m_c$ (by formula (6)), since $m_c(max) < 1\%$, we calculated the values of the average number of induced mutations per initial cell, and, using these averaged figures, constructed the dose-effect curves in Fig. 23a (broken lines), the average number of mutations per 100 cells being given along the ordinate axis. Fig. 23a shows that the results obtained in the two methods fully agree with each other. This confirms the correctness of the premises used as their basis. The small divergences observed may be fully attributable to the imprecision of the selective estimates of $m_a$, $m_c$, n and f. In this same figure it can be seen that $M(m_c)$ are characterized by a lesser degree of variability than $M(m_a)$ which is associated with the differences in the specimen volumes. This once again illustrates the validity of the arguments in favor of $m_c$ presented in Ch. 8.

Thus, the results obtained have shown the effectiveness of the methods adopted for quantitative recording of induced mutations; therefore, these methods were applied to the data of all experiments described in subsequent chapters.

TABLE 9. PIGMENT ANOMALIES OF M2 AND RESULTS OF THEIR ANALYSIS

| Nature of anomalies | Total number | Phenotype | | | | Viability | |
|---|---|---|---|---|---|---|---|
| | | alb | xa | ch | vi | survived | died |
| Mutations | 80 | 9 | 23 | 24 | 24 | 23 (4ch;19vi) | 57 |
| Modifications | 48 | 0 | 0 | 28 | 20 | 20ch 18vi | 8ch 2vi |

Some words in conclusion on the quality composition of the pigmentary anomalies observed in M2. We already said that Table 8 only contains 'proven' mutations, but in addition to these uninherited anomalies (modifications) were also observed in these same experiments. Furthermore, chlorophyllic mutations differed from one another in terms of both their coloration and their viability. Data on all of this are summarized in Table 9.

From the table it is clear that for the experimental data, about 2/3 of all pigmentary anomalies in shoots recorded in M2 were mutations; the remainder were modifications. There were four types of mutations of which albina was the rarest; the others were evenly divided. All alb and xa mutations are lethal, but among ch and vi (especially the latter) viable forms were encountered. No modifications of the alb type were found at all; and among ch and vi, their share was roughly half, 3/4 of these being viable. The sizable number of modifications among pigmentary anomalies of the ch and vi speaks against their being recorded in experiments on mutagenesis. However, the frequency of ch and vi modifications in $M_r$ is not dependent on seed irradiation dosage. /113

| Gamma irradiation dosage, krad | 0 | 10 | 20 | 40 | 70 | 110 |
|---|---|---|---|---|---|---|
| Frequency of modifications, % | 0.12 | 0.03 | 0.31 | 0.10 | 0 | 0.10 |

Consequently, they can only increase the overall background of anomalies, but for all practical purposes subtraction of the control group reduces their contribution to zero, although the dispersion will herein increase.

## 9.5. Concluding Remarks

In this chapter we have treated in detail the somatic and genetic effects observed for gamma irradiation of dormant seeds. Plant loss in the cotyledonous and rosette phases, inhibition of root and stem growth, delay of vegetative and generative development in the plants and a reduction in their ferility are the primary qualitatively well-defined somatic effects of irradiation. The following chapters are devoted to the study of the relationship of these effects as functions of seed irradiation conditions and their comparison in this respect with genetic effects (chlorophyllic and embryonic mutations).

The data presented in Figs. 13-23 indicate that most somatic effects and, to a lesser extent, genetic effects are subject to considerable variability, which cannot be attributed to differences among experimental series in dosimetry or the controlled conditions under which the plants were cultivated. Apparently, the primary cause of the variability observed in the variation in the radiosensitivity of the seed material (established within the field of radiobiology), variability which is dependent on the conditions under which the seeds mature, on the duration and conditions under which they are stored, etc. Therefore, it is advisable to perform all comparisons for dormant seed irradiation with the control group when studying the influence of various factors on somatic and genetic effects of irradiation. All the more so since (as will be seen in following chapters) the variability observed in the experiments on dormant seeds, in which irradiation effects were exhibited, remains for the transition to other irradiation conditions. The results of experiments with high-speed neutrons are an exception. In this case, the irradiation effects vary little both between reiterated experiments within a single series as well as among various experimental series. Lesser variability of the results for seed irradiation by various beam ionizing radiations has been noted repeatedly in the literature (Caldecott et al., 1954). Therefore, in assessing the relative effectiveness of neutrons vis-a-vis gamma-rays, we would be well advised to compare the effects of neutron irradiation not with the results of individual tests using gamma irradiation, but with the average values of said effects. /114

## Chapter 10. The Effect of Gamma Irradiation on Swelling and Swollen Seeds

### 10.1. General Remarks

The increased radiosensitivity of plant seeds during the transition from a state of rest to germination is a fact long known. It was established qualitatively in the first work on plant radiobiology published in 1896 (Schorber), although there was no dosimetry at that time and Schober's experiments were far from perfect methodically. In 1904-1920, numerous experiments of Koernicke were published in which this fact was conclusively verified qualitatively and quantitatively on seeds and sprouts of many plant species and with the application of various X-ray intensities and radium emissions as well (Koernicke, 1904, 1905, 1915, 1916, 1917). And all subsequent radiobiologic experiments on plant seeds showed the same thing. It is not expedient to discuss the vast literature on this subject. It can be found in periodically appearing reviews in the references (Stadler, 1930; Breslavets, 1946; Ehrenberg et al., 1953; Davidson, 1964).

But different aspects of the change in radiosensitivity of seeds in their swelling and sprouting process are still being discussed in contemporary radiobiologic literature (Caldecott, 1954; Ehrenberg, Gustafsson, 1954; Konzak, 1955; Poryadkova et al., 1960; Heaslip, 1963; Nuzhdin, Filev, 1965; Al'shits, 1969; Abdalla, Roberts, 1969). This has to do with the overall picture being clear, while its individual essential features still remain poorly studied. In particular, the enumerated works show that the same degree of the seeds' swelling (especially at the start of this process) can be of unequal importance for the various radiobiologic reactions. This circumstance is especially interesting on the plane of intercomparing the various radiobiologic and radiation genetic reactions. /115

As it has already been noted above, irradiation of not only dormant but of swelling seeds also was used in several radiobiologic and radiation genetics experiments on arabidopsis. In particular, premoistened seeds were exposed to radiation in most experiments on radiation mutagenesis, starting with the first work of E. Reinholz (Reinholz, 1947).

Furthermore, experiments were performed on arabidopsis by various authors in which the effect of seed swelling on their radiosensitivity was one of the main research problems.

For the most part, these were experiments where the dormant seeds' radiosensitivity was compared to that of fully swollen seeds (over 6 hours of swelling at room temperature). These experiments revealed that the swollen seeds were distinguished from dormant by a much greater radiosensitivity, identifiable by

such features as delayed germination (Devi et al., 1964; Contant, 1966); depressed growth and development (Contant, 1966); reduced fertility (Contant, 1966), a common rate of survival (McKelvie, 1963; Contant, 1966; Fujii, 1967, 1968) and the frequency of different type induced mutations (McKelvie, 1963; Veleminsky et al., 1964; Fujii, 1967, 1968), i.e., by all the basic irradiation somatic and genetic effects. While qualitatively similar results are observed when X-rays (McKelvie, 1963; Devi et al., 1964; Veleminsky et al., 1964), gamma rays (Fujii, 1967, 1968) or different energy-level neutrons (Contant, 1966; Fujii, 1967, 1968) act on seeds. True, the seeds' sensitivity to 14-MeV neutrons after 24 hours of swelling increases less than it does to $Cs^{137}$ gamma rays. Therefore, the neutrons' RBE rated by $LD_{50}$ values was 10 for dry seeds and a total of 5 for swollen seeds (Fujii, 1968).

Some experiments have been carried out on arabidopsis where the dynamics of change in the seeds' radiosensitivity during the swelling process was studied. Thus, the experiments of Roebbelen (1962a, 1965) revealed that in exposing swelling arabidopsis seeds to X-rays (a 16 krad dose), the chlorophyllic mutation frequency increases steadily from the onset of absorption to 24 hours and remains constant after this up to 36 hours of soaking. But in A. Mueller's similar experiments (1966a), the embryonic lethals frequency at 8 and 72 krad doses did not change until the seeds' water content reached 22%, which corresponds to their full swelling; when the seeds' water content was from 6 to 16%, the occurrence of induced embryonic lethals remained constant. True, the /116
same author discovered that with anaerobic soaking of seeds (Mueller, 1965a), the occurrence of induced mutations (12 krads of X-rays) only increased during the first two hours of swelling and then becomes stabilized. In this way, the nature of change in seed radiosensitivity during the swelling process may depend on the soaking conditions and probably the discrepancy in Roebbelen's and Mueller's results from this.

R. Contant studied the change in radiosensitivity of arabidopsis seeds during the swelling process at greater length in experiments on irradiation of seeds with high-speed reactor neutron fractions (Contant, 1968). In his experiments, dry seeds, swelling seeds within 20 minutes and 1, 3, and 6 hours before the start of soaking up to the start of germination were exposed to radiation. In all cases the dose-effect curves were obtained. It was discovered that irradiation of swelling seeds (20 min. to 1 hour of soaking) versus neutron irradiation of dry seeds, sharply reduces the total number of ovules in pods and the portion of those fertilized among them, and increases the frequency of embryonic lethals; the utmost swelling of seeds to sprouting does not increase their radiosensitivity estimable by these features.

All information on changes in radiosensitivity of arabidopsis seeds during swelling is limited to the work discussed.

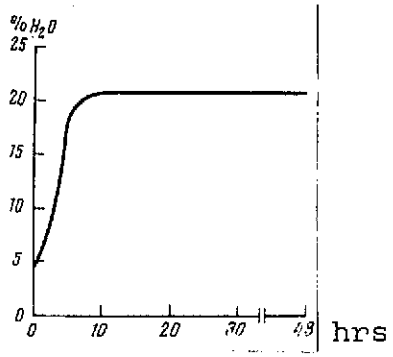



Fig. 24. Curve of swelling of arabidopsis seeds

As we can see, the number of irradiation effects studied is small (sterility and mutation) and dose curves have only been obtained in Contant's high-speed neutron experiments.

At the same time, these examples show that arabidopsis seeds, like those of other plants, have sharp changes in sensitivity during the first hours of swelling; moreover the maximum occurrence of different reactions may be reached at a different time after the onset of seed soaking. This implies that comparing the different somatic and genetic effects of irradiation of seeds at different periods of swelling may prove to be not without interest. The experiments set forth below are devoted to this question. A brief presentation of the basic results of these experiments can be found in the work of Ivanov et al. (1969, 1969a).

## 10.2. Experimental Techniques

The present experiments, with respect to material, plant cultivation techniques, and the recording of various features, did not differ from those for gamma irradiation of dormant seeds set forth in the preceding sections. The main difference between them consisted in the swelling and swollen seeds also being irradiated in addition to dormant seeds. Since the dose-effect curves for all features studied (which were plotted along six dose points here) were the basis of comparison as in the previous experiments, the number of swelling variants had to be limited to the bare minimum. Proceeding from what was discussed above, comparing the different effects of dormant seeds with those of swelling and completely swollen seeds had the most interest. The swelling dynamics of En-1 test-race seeds were studied to determine the presoaking period necessary. The results obtained showed that at room temperature, the seeds' water content increases rapidly (in about 6 hours) from the original 5-6% up to 20-22% in dormant seeds; it then retains this level until mass sprouting observed on the average within 48 hours after soaking starts (Fig. 24). It was found in reiterated tests that the swelling dynamics of the seeds are subject to considerable variation. /117

Thus, in order to avoid the seeds' physiologic condition being greatly different by the time of irradiation the median of the curve's rising segment, i.e., 3 hours after soaking began. and the period between the onset of soaking and mass sprouting (24 hours) were selected as swelling variants. That the seeds' swelling had already begun but not yet finished could be ensured

by the first and that their swelling had been completed but not their sprouting by the second. The seeds were soaked in both cases under aerobic conditions between layers of wet filter paper. Dormant seeds were also irradiated in all the experiments of this series in addition to swelling and swollen seeds. The necessity of this results from the tolerable variability noted above (Chapters 6 and 9) of the majority of irradiation effects studied.

The seeds were irradiated in a cobalt-source "Gamma Cell-220" unit. The dose was 200 krads/hour. It was found that the maximum acceptable irradiation dose of swollen seeds and sprouts was 40 krads and at the same time it became clear from previous ex- /118
periments that the dose range had to be wide enough for a one-time study of the different effects. Therefore, the following doses were adopted in the experiments under consideration: 0, 2.5, 5, 10, 20 and 40 krads.

The following somatic features were recorded in the M1 generation: the seeds' germinating capacity, the plants' survival rate in subsequent stages of development, the main root length on the 7th day of the experiment, the rosette lobes appearing by that day, the raceme lobes appearing by day 21 of the experiment, the average number of seeds per pod and the frequency of sterile plants among the survivors. Chlorophyllic mutations among the M2 sprouts were also recorded.

A total of 10 experiments were conducted in this series with the total volume of material in M1: 20 seeds per variant x 6 irradiation doses (including the control group ) x 3 swelling variants x 10 experiments = 3,600 test seeds. To keep the chlorophyl mutation record in M2, 175,920 sprouts were grown. The results obtained in the experiments were processed statistically as usual.

## 10.3. Test Results and their Discussion

The results obtained in the experiments are presented in figures 25-29. As in the preceding chapter, when plotting the dose-effect curves, the values of all somatic features are stated as percentages (with the non-irradiated control group subtracted) and the average per 100 cells (with the control group subtracted) is computed for chlorophyll mutations. All experiment points in figures 25-29 are shown with their errors calculated as compound average errors. As was to be expected, the soaking of non-irradiated seeds did not affect the plants produced from them in any way. Therefore, all deviations were computed on the average value of the three control versions (without irradiation). Since no irradiation effect was observed on the seeds' germinating capacity, as in other series of tests, and the death of plants in later stages of development was

negligible, data regarding these features were not presented in the graphs.

Looking at the results obtained as a whole, it can be noted that in the present series of experiments, dormant seeds proved to be more sensitive to gamma irradiation from nearly every feature than in the other experimental series (Ch. 9). Such an increase in radiosensitivity of the seeds cannot be attributed to differences in irradiation, dosimetry or the test plants' cultivation conditions. In these respects, strict uniformity was observed in all test series. The sole difference among the test series is that various batches of seeds were used as the test material and this may be extremely significant as we know from radiobiology. It is impossible to conduct perennial experiments on arabidopsis with one batch of seeds since the seeds being losing their germinating capacity about two years after harvesting. Moreover, the limits within which the seeds' radiosensitivity may vary are not without interest for the general radiobiological features of arabidopsis. It is evident from figures 25-29 that, as in tests on /119 dormant seeds, increasing the irradiation dose of swelling and swollen seeds earlier only affects the plant's growth and fertility, afterwards a marked delay in vegetative and generative development is observed and finally, the plants' death--at first in the rosette stage, then in the cotyledon stage, and all these somatic effects are accompanied by growth with an irradiation dose of an induced mutation amount.

Of course some partial distinctions of dose curve forms from those discussed in the previous section can be discerned. However there are not enough grounds for a detailed analysis of curve forms derived under various conditions for the features subject to tolerable variability. In the present case, something else is of greater interest: how the emergence of the somatic and genetic reactions to be compared was changed in switching from irradiation of dormant seeds to swelling and swollen seeds. The results of statistical processing of data obtained, which are shown in figures 25-29 and table 10, as well as the values of the dose change factor (DCF) for all the features studied can be used as the basis for such comparisons.

The DCF values were calculated here and henceforth as the average ratios of isoeffective doses in the compared versions of the experiments. In addition, the results of dormant seed gamma irradiation always served as the standard. The mean dose values, according to data cited in Ch. 9, were used for some features, whose changes were negligible in radiation doses of dormant seeds up to 40 krad (the sprouts' survival rate in the cotyledon stage, sterility), but with a correction for the deviations in the results of this series of tests from the mean dose-effect curves for the appropriate features.

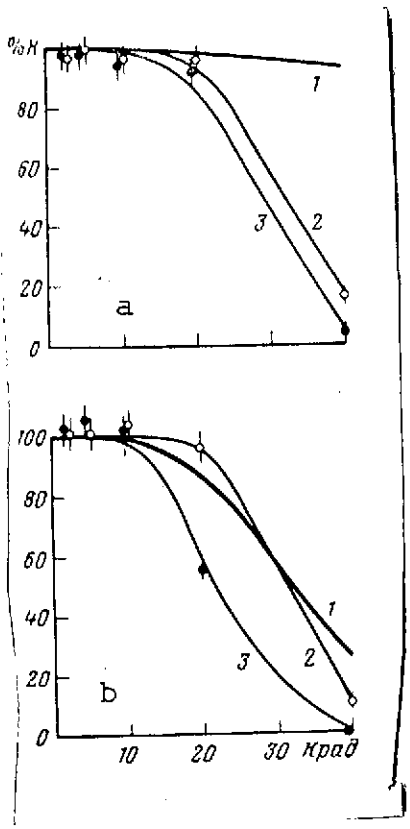



Fig. 25. Survival of sprouts in phases of cotyledon (a) and rosette (b) at different doses of gamma-irradiation. (1) irradiation of dormant seeds; (2) swelling [3 hrs, light dots]; (3) swollen [24 hours, dark dots].

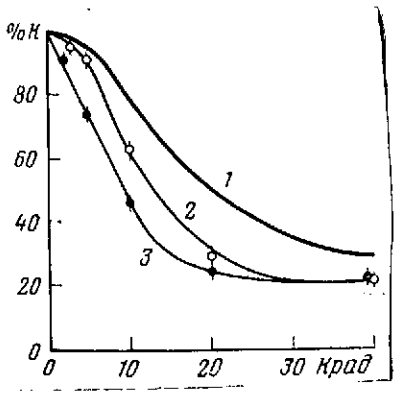
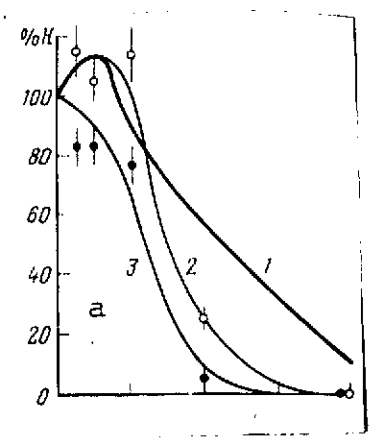
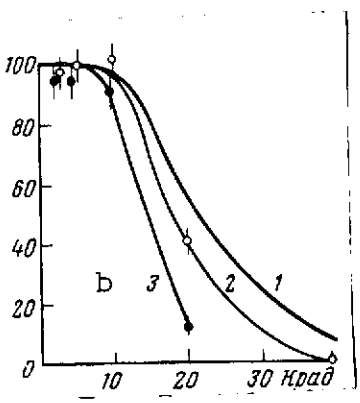



Fig. 26. Root length on Day 7 after sowing at different doses of gamma-rays (Notation as in Fig. 25).






= KRAD

Fig. 27. Rate of Vegetative (a) and Generative (b) Development of Plants at different doses of Gamma Irradiation (Notations as in Fig. 25).

/120

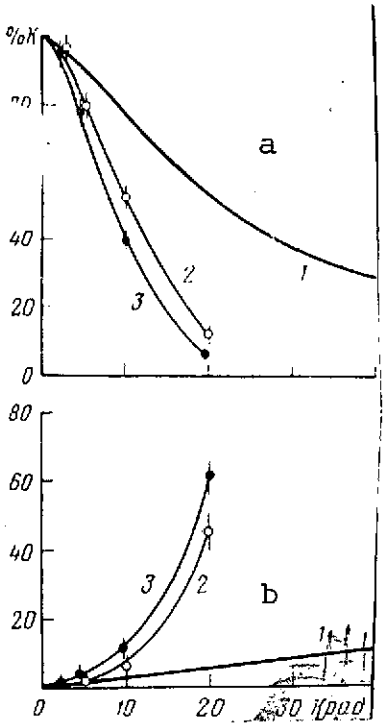



Fig. 28. Number of seeds in pods (a) and frequency of sterile plants (b) at different doses of gamma irradiation (Notations as in Fig. 25).

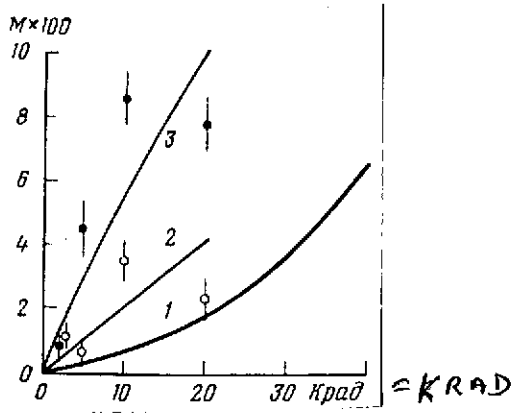



Fig. 29. Average number of mutations (M) per 100 cells at different doses of gamma-irradiation (Notations as in Fig. 25).

If the different final effects observable with various radiation doses had had the same cellular damage mechanism as their basis, then an identical (or at least an analogous) realtion of all the effects and the seeds' stage at the time of irradiation could have been expected. Then the formation of the different final effects could have been considered the result of purely quantitative differences in the degree of damage of irradiated meristems, differences determined first by the absorbed radiation dose and the in the same dose by the variability of its distribution through and inside the cells; by the the seeds' individual radiosensitivity; and so forth.

But figures 25-29 and Table 10 show that the case is somewhat different: irradiation effects are changed in different ways according to the seeds' state at the time of irradiation. It is common to all the reactions herein that their occurrence increases substantially in the irradiation of fully swollen seeds (24 hours) versus dormant seeds. True, in addition to this, some quantitative differences in the degree of the effects' increase /121 may be noted: such effects as plant deaths during the rosette stage and the delay in generative development increase least of all and induced sterility increases most of all, while the other effects--sprout death during the cotyledon stage, depressed root growth, delayed vegetative development, reduced number of seeds and mutations--are in the intermediate position. In table 10, the relatively low values of the $DCF_{24hr0hr}$ (1.3-5.4) versus the plants'

usually observed 5 to 10-fold increase in seed radiosensitivity resulting from their swelling attract attention. This indicates that the irregularly high radiosensitivity of dormant arabidopsis seeds which has been found in the current experiment series was not extended to swollen seeds, which in turn coincides with the well-known phenomenon in plant radiobiology -- the considerably weaker dependence of radiosensitivity of sprouts and swollen seeds versus dormant seeds on their batch (i.e., on their age and maturation and storage conditions).

A different pattern was observed when seeds were irradiated three hours after soaking. After a 3-hour soaking, unlike the 24 hour one, where all irradiation effects increase substantially, a significant increase was observed only for some of them (the death of sprouts during the cotyledon phase, depressed root growth,/122 reduced number of seeds, induced sterility), while the rest did not differ significantly from irradiation of dry seeds (the death of plants in the rosette stage, delayed vegetative and generative development, mutation formation).

In this way, the action of soaking seeds on the various after-effects of their irradiation are not only shown in how much the recorded radiation effects increase, but in when this increase sets in. If both these aspects of the effect of seed soaking on different irradiation effects were to be taken into account, the following sequence of modifiability of arabidopsis' somatic and genetic effects, when irradiating swelling and swollen seeds, could be constructed:

$$R = G < V < M < C < L < N < S.$$

In this sequence, the least modifiable radiation effects, i.e., those which start their increase late when seeds are soaked and attain lesser values when seeds are fully swollen, are on the left and the greatest, i.e., those which start increasing early and attain the highest values at full swelling are on the right. The meaning of the symbols used is given in Table 10.

The question may arise: is it expedient to describe the act-/123 ion of the 3- and 24-hour seed soaking by a generalized modifiability sequence of the irradiation effects in question? The presence of a positive correlation between the DCF values (3-0 hrs) and DCF(24-0 hrs), i.e., those from the irradiation effects whose increase is observed earlier, as a rule, yield the highest DCF values in the irradiation of fully swollen seeds, speaks in favor of constructing this sequence.

Another question may arise: is it expedient for the comparative modificability characteristic of the studied irradiation ef-

fects with swollen seeds to be limited by the simple ranking of these effects instead of comparing them by numerical DCF values? In connection with the repeatedly stressed variability of the majority of irradiation effects, one may think that although the simple hierarchy of effects is certainly cruder than a comparison of them with numerical DCF values, this is really precisely why it

TABLE 10. THE EFFECT OF PRERADIATION SOAKING OF SEEDS ON THE BASIC SOMATIC AND GENETIC EFFECTS OF GAMMA IRRADIATION IN ARABIDOPSIS

| Effect | Notation | Soaking version, hrs | | | |
|---|---|---|---|---|---|
| | | 3-0 | 24-0 | 3-0 | 24-0 |
| | | P | | DCF* | |
| Death of sprouts in cotyledon phase | C | 0.001 | 0.001 | 1.9 | 2.1 |
| Death of plants in rosette phase | R | 0.05-01 | 0.001-0.005 | 1.0 | 1.3** |
| Depressed root growth | L | 0.001 | 0.001 | 1.6 | 2.4 |
| Delayed vegetative development | V | 0.05-0.75 | 0.001 | 1.0 | 1.7** |
| Delayed generative development | G | 0.5-0.75 | 0.001-0.005 | 1.0 | 1.3** |
| Reduced number of seeds in pods | N | 0.001 | 0.001 | 2.0 | 2.7 |
| Induced sterility | S | 0.001 | 0.001 | 4.3 | 5.4 |
| Occurrence of mutations | M | 0.25-0.5 | 0.001-.005 | 1.0 | 2.9** |

*Dose Change Factor **When $P_{3-0} \geq 0.05$, the DCF = $= \frac{D_{av}(3+0)}{D_{24}}$.

is less fraught with the danger of finding differences where there actually are none.

And, finally, the most serious question: what information about the studied irradiation effects can a hierarchical sequence of their modifiability yield? Of course, the circumstance that some irradiation effect is modified more or less with the seeds'

swelling still says nothing about the nature of the effect nor about the mechanism of the action of the change of an irradiated seeds' water content on the effect. However, the sequence of modificability cited above can be useful for the general radiobiological and radiation genetic description of the arabidopsis and to explain the interrelationship between the different effects on this basis, since it is in such a sequence the effects similar in their formation mechanisms should be placed close to each other, while those dissimilar should be remote.

Of course, it is impossible to rule out the relative placement of the different final irradiation effects in a modifiability sequence--it may be determined not solely nor so much by similarities and differences in their cellular formation mechanisms, but by other features entirely. Thus it may be assumed that the close proximity of any reactions in the modifiability sequence is explained by the reactions under consideration being the result of the damages (irrespective of what type) of the same meristemic cells. It may be assumed that the near proximity of any reactions in the modifiability sequence is likewise determined by the correlativity of appropriate features in the plants' ontogeny. An a priori selection among these three possibilities is difficult, especially between the first two, since correlations among features yield experimental evaluation. As for the first two, it can be expected that in the event of relative placement of various reactions in the modifiability sequence is determined by whether the substratum /124
of these reactions is the same or different cellular material (the second assumption), then the sequence's overall structure should be kept with the action of different modifing factors. If the relative placement of reactions in sequence is determined by similarities or dissimilarities in their mechanisms of occurrence, retention of the general structure of the sequence is not merely optional, but unlikely. In this case, it can only be expcted that reactions similar in formation mechanisms will continue to be drawn toward each other. Therefore, in justification of the first assumption, it should hardly be expected that such different factors as swelling, post-radiative storage of sedds, thermal shocks and radiation LEL (linear energy loss) will yield coinciding or very similar modifiability sequences. Conversely, any radiobiologic reaction which is modified the least under the action of one of these factors may prove to be the least modified under the effect of another. However, this may not hamper exposing the interrelationship among the various irradiation effects. And what is more, if any radiobiological reactions, despite differences in the modifying factors' action mechanisms, are grouped closely in modifiability sequences, one may think that a close interrelationship actually exists among such reactions. And conversely, if any radiobiologic reactions are not positioned closely in modifiability sequences under any (of the studied) conditions, then

one may think that these reactions actually have little in common.

In view of what has been said, it would be premature to make definite conclusions about the interrelationships among the different irradiation effects only on the basis of their changes under the action of one factors, the seeds' swelling.

Moreover, on the strength of the same variability of the features studied, as well as the closeness of the DCF values for some adjacent members of the MS (modifiability sequence) cited above (by the way, this is valid even for MSs based on studying the action of other factors to be discussed in subsequent sections), it can scarcely be thought that any one sequence can reflect the interrelationship among the irradiation effects studied precisely enough. This is why only comparing the interspacing of the different irradiation effects in the sequence under consideration with their placement in analogous sequences derived when studying the modifying action of other factors can serve as the basis for showing their interrelationship.

## Chapter 11. The Effect of Post-radiation Storage of Seeds on the Somatic and Genetic Effects of Gamma Radiation

### 11.1. General Remarks

/125

The change (usually the increase) in the radiation injury of plants during the postradiative storage of dormant seeds prior to sprouting is called the 'storage effect' in plant radiobiology. The study of the effect of postradiative seed storage on the radiobiologic reactions of plants has a long history. In early radiobiologic experiments on plants, storing seeds after irradiation was used to solve the question of the reversibility of damaging action of X-rays, and radium emissions on the seeds' germination and subsequent plant growth (Koernicke, 1904; Guilleminot, 1910). And it had already been discovered then that during postradiation storage of seeds radiation injuries not only survive but can even become more pronounced. And since 72 different plant species were investigated in the experiments, it was possible to think that the increase in the seeds' radiation injuries in the process of their postradiation storage, i.e., what became called the storage effect later on, is a rather common effect.

However, subsequent works made it clear that in experiments on the same irradiated material, the storage effect may affect some radiobiologic reactions of plants and not affect others. Thus, in J. Stadler's experiments (Stadler, 1930), the two-week postradiation storage of barley and corn seeds increased the frequency of chlorophyll mutations but did not affect the germination nor the growth rate of the plants they produced. In A. Gustafsson's experiments (1937), the storage of irradiated barley seed from 4-66 days merely increased the frequency of chromosomal fragments, but without affecting the occurrence of other types of chromosomal aberrations. In A. S. Afanasyeva's (1936) similar experiments on wheat seeds, no storage effects were found at all. Moreover, both in the experiments just mentioned and others it was shown that if the storage effect succeeded in being observed, then it was usually negligible in magnitude--a couple of dozen percentage points. The small magnitude of this effect and its great variability led to plant radiobiologists losing interest in the storage effect for a while. They only regained interest in the 1950's and 1960's, when the period of intensely studying the modification of the biologic effect of ionizing radiation began in radiobiology in general (and in plant radiobiology in particular).

/126

By now, the total number of publications particularly devoted to the study of the storage effect already exceeds 100. Reviews of the primary results of experiments on storage effects and bibliographic information can be found in the works

of Ehrenberg (1956), Ehrenberg, Lundquist (1957), Caldecott (1961), Konzak et al., (1961), Khvostova, Nevzgodina (1962), Berezina (1964), Abdalla, Roberts, (1969), Sanina, (1970).

The net result of storage effect study is as follows: an increase in the radiatio injury of seeds during this postradiation storage can be seen in the seeds of any plant species and in any radiobiologic reactions, but in each case the conditions favoring the appearance of the effects must be found. Under the favorable conditions which are discussed below, the storage effect can be observed in the most diverse radiobiologic plant reactions (death, depressed growth and development, sterility, various types of mutations). True, such an affirmation has to be made, generalizing the results of the many authors, each of whom studied one or most likely two reactions. These are mainly the chromosome aberrations in the first mitoses after irradiation or delayed first leaf growth in grains. There are very few works in which the storage effect's appearance in several radiobiologic reactions of plants would be studied one time and under the same experimental conditions (Adams et al., 1955; Adams, Nilan, 1958; Caldecott, 1958; Nilan et al., 1961; Lebedeva et al., 1966; Kawai, Sato, 1966; Pomogaybo, 1968, 1969). This is why it is difficult to assess the storage effect's role in various radiobiologic reactions.

The variability of the storage effect's appearance noted in the earlier experiments was also confirmed and the main reasons for it have been studied quite well. It was found that the appearance of the storage effect was related in a complex way to the whole complex of conditions of conduct of radiobiologic experiments on plant seeds.

First of all, the storage effect frequently observed in experiments with gamma- and X-rays is absent apparently under the influence of hard ionizing radiations on seeds (in biologically comparable doses)(Ehrenberg, 1956; Caldecott, 1958; Nilan et al., 1961; Nuzhdin et al., 1963; Khvostova et al., 1965). Some authors did observe the storage effect after seeds of different plants were irradiated by thermal and fast neutrons (Yagyu, Morris, 1957; Shkvarnikov, Chernyy, 1964; Pomogaybo, 1968, 1969). But these observations were not confirmed in similar experiments of most other authors. In our experiments on irradiation of dormant arabidopsis seeds with 25.1 MeV alpha particles and 5.6 MeV neutrons, the storage effect was likewise unconfirmed.

Another major factor defining the appearance of this effect /127 is the length of storage after irradiation. By tracing the storage effect dynamically, two of its components, the fast and the slow, succeed in being isolated. The fast component begins to appear several hours after irradiation and increases

to one or two days, whence the time-effect curve bends, but does not level out on a plateau; it continues to rise slowly until several weeks or even months after irradiation. In still greater storage periods, the occurrence of radiobiologic reactions is stabilized and may even vanish (Dishler, 1965; Berezina et al., 1966; Semerdzhyan et al., 1968). But even with respect to the storage dynamics, the data of various authors is quite contradictory.

Apart from the basic factors discussed, the occurrence of the storage effect also depends on the age and physiologic condition of the irradiated seeds, their water content, the irradiation and storage conditions, the temperature range prior, during and after irradiation, the gas composition of the environment, the soaking conditions and the plants' growth. In short, it is hard to name one factor which would not affect the occurrence of a storage effect. The complicated relationship of the storage effect and the entire set of factors and conditions in radiobiologic experiments on plant seeds produced tolerable inconsistencies in the results--particularly in their interpretation. This is why no single satisfactory explanation for this effect has been yet found. Attempts which seemed fruitful at one time to relate the storage effect to an oxygen effect and aftereffect (Caldecott, 1961) or with the action of independent radicles (Zimmer et al., 1957) met with the experimentors' strong objections later on.

Therefore, it can be considered adequately reliably established only that in some cases with irradiation of plant seeds, the development of radiation injury may be prolonged even into the postradiation period; this circumstance must be taken into account when planning radiobiologic experiments.

As concerns arabidopsis, the information on the plant's 'storage effect', apart from experiments set forth in this chapter, is limited to A. Mueller's data (1967) that after a 12 kR dose of X-ray radiation on anaerobically soaked seeds and their subsequent drying and storage for 75 days, the frequency of embryonic lethals and chlorophyllic mutations increased; Contant and Dankert (1968) found that there is no 'storage effect' when storing dormant seeds irradiated with various doses of gamma-rays and fast neutrons at -20°C for 18-53 hours.

It thus became important to evaluate the importance of the 'storage effect' in the radiobiologic reactions of arabidopsis. Another goal of such experiments was to compare the main somatic /128 and genetic effects found in arabidopsis with respect to postradiation storage of its seeds.

The primary findings of the experiments on the storage effect in arabidopsis by the author and his collaborators have been pub-

lished (Ivanov et al., 1969b; Sanina, 1970; Sanina et al., 1970).

## 11.2. Experimental Techniques

All experiments set forth in this section were performed according to standard procedures described in detail above (Chapters 4-6, 8, 9). The basic difference consists in some of the material's being irradiated two weeks prior to the experiment and being stored that length of time at room temperature. The post-radiation storage period--two weeks--was selected on the basis of the findings of special preliminary experiments (Ivanov et al., 1969; Sanina, 1970; Sanina et al., 1970), in which En-1 dormant seed were sown after irradiation by 70-krad $Co^{60}$ gamma-ray doses in 0, 1.5, 3 and 12 hours and 1, 4 and 16 days. These tests proved that up to 12 hours after irradiation, no differences are found in freshly irradiated and stored seeds; in the 12-hour to 4-day period (for various reactions in different ways), the increase in the storage effect is observed; after storing the seeds 16 days the occurrence of reactions is the same or only slightly higher than after 4 days of storage. Of course a study of the dynamics of storage effects in various reactions and at several reactive irradiation doses would be most informative. But the planning of such experiments would include too many variables: a minimum of 8 reactions X 3 or 4 irradiation doses X 3 to 4 storage periods, i.e., at least 72 versions. That is why it was decided to obtain dose curves for the basic somatic and genetic irradiation effects only in one storage period, where a rather complete appearance of the storage effect could only be anticipated after a comparison of these data with those for freshly irradiated seeds.

The seeds were irradiated in a $Co^{60}$ "Gamma-Cell 220" at the NIIMR AMN SSSR (Scientific Research Institute of Medical Radiobiology of the Academy of Science USSR) with about a 200 krad per-hour dose. The following features were recorded: the seeds' germinating capacity, the survival rate of plants in the basic development stages, root length on Day 7 after onset of sprouting, the height of plants on the day blossoming starts, the duration of the sprouting-rosette-budding-blooming stages, the total number of seeds/pod and the number of sterile ovules and mutant seeds in them (embryonic lethals and chlorophyll mutations). Remembering that the storage effect is variable, it was decided to conduct two independent test series under the same conditions for subsequent analytic comparison. In addition, since the various effects of arabidopsis irradiation are exhibited in /129
different dose ranges, each series was split again into two groups of tests--one with small doses, the other with large--to save time and material. Every test in all series and groups included 9 versions: a nonirradiated control group and 4 each with irradiation doses without storage and with 2 weeks storage, with 40 seeds per version. In the first series with

small doses (10, 20, 40 and 80 krads), five tests were run and three at large doses (50, 100, 150 and 200 krad). In the second series, six tests were run with small doses (20, 40, 60 and 80 krad) and 5 at large doses (50, 100, 150 and 200 krad). Thus the total number of M1 test seeds was: 40 seeds per version x 9 versions x (5 + 3 + 6 + 5) = 19 tests = 6,840 seeds; and the total number of M2 recorded seeds in all tests was 304,290. The first 5 pods of the main fruit system were employed to record fertility, sterility and frequency of mutations.

In Chapter 6 the statistical methods of experiment planning and processing of results are discussed.

11.3. The Results and Discussion of the Tests

The summary data on the effect of the two-week postradiation storage of seeds on arabidopsis survival rate, growth, development, feriility and frequency of mutations are cited in Figures 30-34. Moreover, as in the preceding section, only the dose curves for the main somatic and genetic effects are shown therein, expressed in percentage points of the control group. The limits of error are given for all experiment points. In the small-dose tests (up to 80 krad), neither irradiation nor postradiation storage affected seed germinating capacity and survival rate of sprouts in the cotyledon stage or of plants in the generative stage. At a 80 krad dose in both test series, some reduction in survival rate of plants was observed in the rosette stage (especially in versions using stored seeds), but this reduction was negligible. This is why the data obtained at small doses was not employed to assess the effect of seed storage on the survival rate of the plants. The data obtained at large irradiation doses suits this purpose far better. The plants' growth, development and fertility features changed considerably and significantly at small irradiation dosages (up to 80 krad) for arabidopsis. The only exception was the length of the budding-blooming stage: a significant but negligible change was observed at 40- and 80--krad doses in the first test series. The mild effect of seed irradiation on the length of the later stages of development is generally typical of arabidopsis (Nikolov, 1968; Nikolov, Ivanov, 1968). The frequency of embryonic and chlorophyll mutations increased substantially with the irradiation dose in both series.

It is evident in figures 30-34 that the shape of the dose /131
and effect curves (allowing for variation in somatic features) reiterated the corresponding curves shown in the preceding sections adequately. Just as in the previous tests, with an increase in irradiation dosage the plants' growth was first depressed and fertility reduced; then their development began to lag markedly and only at the highest doses was the lethal

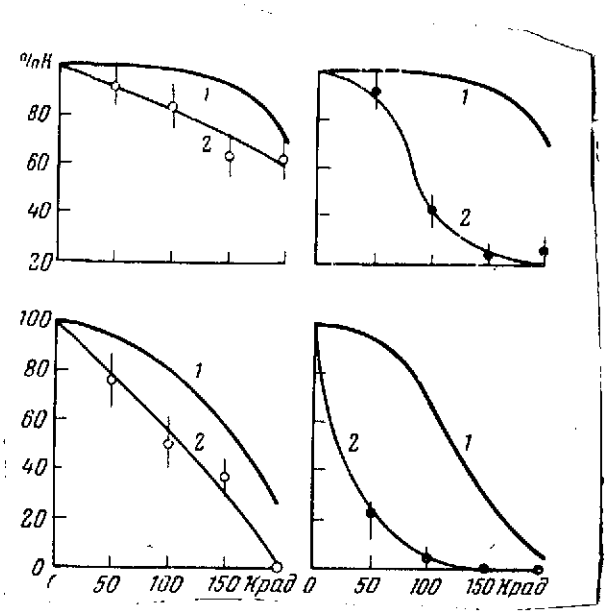



Fig. 30. Survival Rate of Sprouts in phases of Cotyledon (top) and Rosette (bottom) at different doses of seed gamma irradiation. 1) sown right after irradiation; 2) after 2-week storage at room temperature. Left: Series I, right: series II.

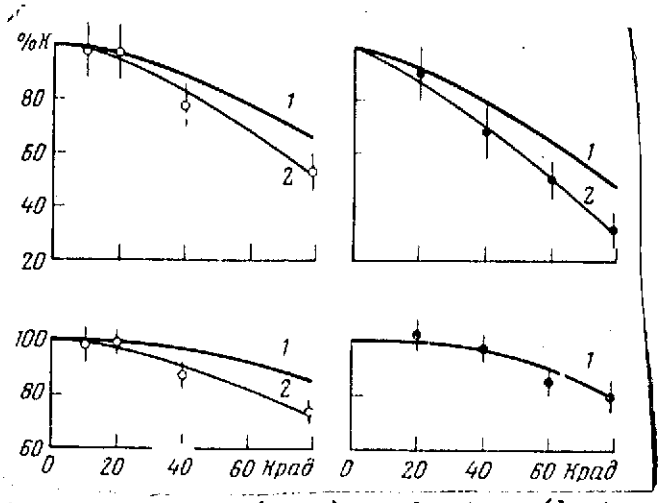



Fig. 31. Root (top) and stem (bottom) growth at different doses of seed gamma irradiation (Notations as in Fig. 30).



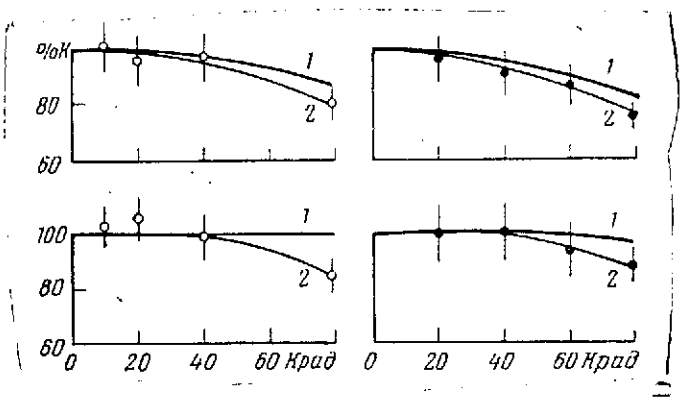



Fig. 32. Rate of Vegetative (top) and Generative (bottom) Development of Plants at Different Doses of Seed Gamma irradiation (Notations as in figure 30).

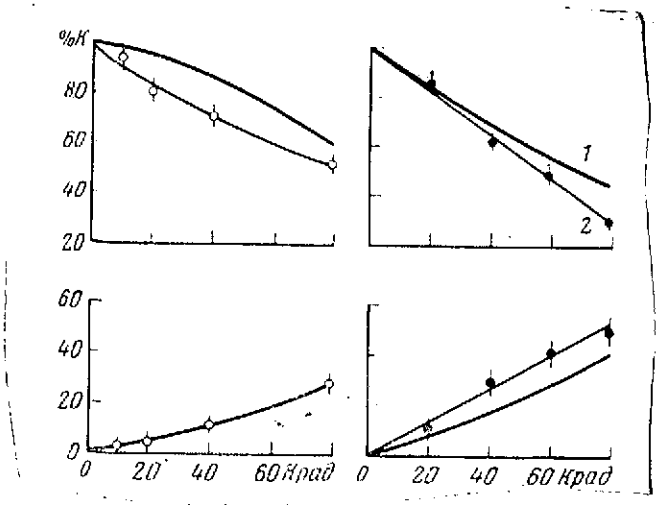



Fig. 33. Number of seeds in pods (top) and Frequency of Sterile Seed-buds (bottom) at Different Doses of Gamma Irradiation of Seeds (Notations as in Fig. 30).

effect, confined to the early life-cycle stages, observed. All these somatic effects of irradiation were accompanied by the growth in the number of induced mutations.

Regarding the storage /132 effect, as might have been expected, its appearance varied within tolerable limits. As can be seen in figures 30-34 and Table 11, where DCF values and the basic statistics are given, a distinct and significant storage effect was found in both test series for only four of the nine basic parameters



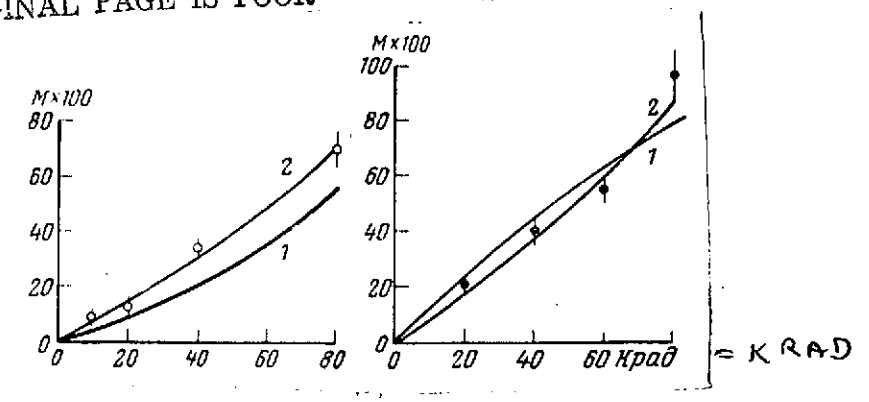



Fig. 34. Average Number (M) of Embryonic Lethals and Chlorophyll Mutations per 100 cells at different doses of Gamma irradiation(Notations as in Fig. 30).

(death of sprouts in the cotyledon phase, death of plants in the rosette stage, depressed root growth and reduced number of seeds). For the other four features (depressed stem growth, delayed vegetative and genetic development and the number of mutations) a significant increase in radiation inury during storage of irradiated seeds was noted only in the first test series. Finally, the storage effect was only taken for induced sterility in the second series. Meanwhile, these series of experiments did not differ from

each other in material or procedure. The only difference in their procedures consisted in their being conducted at different times, since it took about a year to carry out all the experiments.

TABLE 11. THE EFFECT OF POSTRADIATION STORAGE OF SEEDS ON THE MAIN SOMATIC AND GENETIC EFFECTS OF GAMMA IRRADIATION IN ARABIDOPSIS

| Recorded Effect | Notation | Effect of storage | | | |
|---|---|---|---|---|---|
| | | P | | DCF | |
| | | Series I | Series II | Series I | II |
| Death of sprouts in cotyledon phase | C | 0.01-0.025 | 0.01-0.025 | 2.6 | 3.3 |
| Death of plants in rosette phase.. | R | 0.001 | 0.001 | 1.9 | 3.1 |
| Depression of root growth..... | L | 0.001-0.005 | 0.005-0.01 | 1.3 | 1.4 |
| Depression of stem growth..... | H | 0.001-0.005 | 0.25-0.5 | 1.5 | 1.0 |
| Delay in vegetative development..... | V | 0.01-0.025 | 0.1-0.25 | 1.2 | 1.0 |
| Delay in generative development..... | G | 0.001-0.005 | 0.1-0.25 | 1.3 | 1.0 |
| Drop in number of seeds in pod | N | 0.01-0.025 | 0.001-0.005 | 1.7 | 1.1 |
| Induced sterility ..... | S | 0.25-0.5 | 0.001-0.005 | 1.0 | 1.5 |
| Mutation occurrence ..... | M | 0.01-0.025 | 0.5-0.75 | 1.4 | 1.0 |

Thus, the storage effect in tests on arabidopsis, like those on other plants, can be observed in all the basic radiobiologic reactions, and, as in other plants, its appearance is unstable in arabidopsis. The similarity with other plants extends to the magnitude of the storage effect, which can be visualized by the DCF values cited in the last two columns of Table 11. Since no interaction is detected, as a rule, between the irradiation and storage effects, the DCF values were calculated as the average ratios of isoeffective doses. In Table 11 it can be seen that the DCF values for various irradiation effects differ somewhat from each other, and for the majority of effects the DCF magnitudes in both test series did not exceed 2 and only in lethal effects were DCF /133 values on the order of 2-3 obtained.

It is notable that a positive correlation was detected between the sets of DCF values derived according to the results of the two independent test series. This indicates the lack of randomness of variation in DCF values within each set, i.e., the re-

ality of differences in terms of 'storage effects' in various radiobiologic reactions of arabidopsis. Since these two series of tests to study the 'storage effect' did not differ from each other in terms of material and procedures, there is no basis to give preference to either of them; therefore, the average DCF values of the two test series may be used as the best estimates of the magnitude of the storage effect. If the radiobiologic reactions examined were to be placed in a sequence of storage effect increase, we would obtain the following modifiability sequence:

$$V < G < M < H = S < L < N < R < C.$$

Without reiterating the considerations expressed at the end of the last section on the possible use of a modifiability sequence to analyze the interrelationship between these effects and the need for such analysis to compre sequence obtained in studying /134 the effect of irradiation of different modifying factors on the somatic and genetic effects, we will set aside the discussion of the question of this interrelationship until Chapter 14.

In concluding, let us note that all the aforementioned facts on the storage effect with irradiation of arabidopsis seeds is related to the effect's so-called fast component, i.e., to the processes which are induced by the occurrence of unstable biologic active products of primary radiation chemical reactions in the irradiated seeds. Generally speaking, the word 'storage' is poorly suited for the fast component of the storage effect, since in Russian it is associated with long-term periods: and what are two weeks to dormant seeds? Perhaps it would be better to call the fast component of the storage effect an 'irradiation aftereffect' leaving the term storage for the slow component induced by rather different factors: the accelerated aging of irradiated seeds. Indeed, this interesting phenomenon has not been studied at all in arabidopsis.

## Chapter 12. The Effect of Thermal Shocks on Somatic and Genetic Effects of Gamma Irradiation

### 12.1. General Remarks /134

Temperature may have been one of the first factors whose possible effect on various aftereffects of irradiation of living organsims drew the attention of radiobiologists. Interest in radiobiologic temperature experiments is linked with specific temperature effects and their absence can produce information on the nature of the reactions studied.

It is thus no surprise that radiobiologists have run many experiments on the effect of different temperatures (increased and reduced, copious cooling and thermal shocks) employed before, during, and after irradiation on the occurrence of the most diverse radiobiologic reactions in all sorts of living organisms.

A detailed discussion of just the question of the temperature factor's importance in plant radiobiology would take up too much /135 space. The basic information on this can be found in reviews (Breslavets, 1946; Ehrenberg, 1956; Konzak et al., 1957; Evans, 1959; Eydus, Ganassi, 1960; Caldecott, 1961; Nikolov, 1968; Timofeyev-Resovskiy et al., 1968; Abdalla, Roberts, 1969).

It seems difficult to take total stock of all the experimental findings on the effect of increased and lowered 'physiologic' temperatures on irradiation effects during seed irradiation, their postradiation storage and germination and during subsequent plant cultivation, since the effect of the same temperatures at different stages of radiobiologic experimentation on plants often proves to be antipodal. In addition to this, the temperature effect usually depends on several other irradiation-associated factors and conditions of the experiment. In tests on plant seeds, first there are such factors as the composition of the atmosphere and the moisture content of the seeds.

The results of experiments on the effect of superlow temperatures (liquified gases) and on irradiation effects of thermal shocks are more specific. Thus it may be considered firmly established that superlow temperatures used during seed irradiation and their postradiation storage reduce the occurrence of the plants' radiobiologic reactions markedly. As for thermal shocks, the nature of their effect depends on whether they are employed prior to or after seed irradiation (Smith, Caldecott, 1948; Caldecott, Smith, 1952; Konzak et al., 1960; Bergbusch, Caldecott, 1963; Shapiro, Protopova, 1964; Shkvarnikov, Chernyy, 1964; Kulik, 1965; Mostafa, 1965; Santos, 1965; Usmanov, 1966; Atayan, 1968). All authors who employed preradiation thermal shocks in their experimentation are unanimous that such shocks reduce the radiation injury of plant seeds and that this is not connected with

the general change in seed moisture content, since the effect of shocks can be maintained for a long time during which seeds recover their original moisture content. At the same time, it is well known that the screening effect of preradiation thermal shocks may be removed by soaking and then drying the seeds slightly prior to irradiation. In view of this, some authors feel the screening effect of preradiation thermal shock to be due to removal of locally fixed water from areas sensitive to irradiation and the original state of water in the seeds may be recovered only when they are wetted. It is also impossible to preclude that the screening effect of preradiation thermal shock may be connected with the removal of oxygen from the seeds and also with the configurative changes in macromolecules and mycellae affected by high temperature.

The situation with postradiation thermal shock is somewhat more vague: in the experiments of most authors cited above, such /136 shocks increased the seeds' radiation injury; yet data were also obtained on the screening effect (Santos, 1965; Atayan, 1968). This inconsistency is partially connected with not only the magnitude but also the direction of postradiation thermal shock effect being a function of several factors and experiment conditions (in particular, of the presence or absence of oxygen during seed soaking) which are different inevitably in the experiments of different authors. The complex relation of the effect of postradiation thermal shock on irradiation effects and the experiment conditions does not make it possible to construct a consistent picture of the mechanism of their action in radiobiologic reactions of plants for the time being.

In experiments on irradiation of plant seeds using thermal shock, most authors studied its effect only on some single (very seldom two) radiobiologic reaction of plants: usually this was the chromosomal aberrations in the root meristem cells and/or first leaf growth (in grain). There are virtually no experiments in which the effect of thermal shocks on all radiobiologic reactions of plants would be studied. And yet, although many radiobiologic experiments employing pre- and post-radiation thermal shocks were carried out (the main ones are cited above) there still were no data obtained on the interrelationship between certain shock types.

In radiobiologic experiments on arabidopsis, the temperature effect has still only been poorly studied. It has been shown (Daly, 1960) that a supraoptimal temperature (27°C) when growing plants from seeds irradiated with 150 kR $Co^{60}$ gamma ray reduces the survival rate of plants: 11% versus 76% at a 20°C cultivation temperature. It is also known that X-ray irradiation of seeds at the temperature of liquified air improves their germinating capacity versus irradiation at room temperature (Reinholz, 1962).

Finally, it was shown that soaking seeds at a reduced temperature (4°C) before their germination favorably affects the kinetics of germinating irradiated seeds (36- and 72- kR X-rays or gamma rays; Kucera, 1966a).

Taking all this into account, the experiments set forth in this section aimed at studying the effect of preradiation, post-radiation and twofold (pre- and post-radiation) thermal shocks on the basic somatic and genetic effects observed in arabidopsis due to gamma irradiation of seeds were planned. The primary results of these experiments are cited in the works (Nikolov, 1968; Nikolov, Ivanov, 1968, 1969).

## 12.2. Experimental Techniques

All experiments set forth in this section were run, as usual, on En-1 A. thaliana race seeds and according to standard procedures described before (Chapters 4-6). The main difference in these /137 experiments is that some of the seed material was exposed (in addition to gamma rays) to the effect of thermal shocks either prior to or after irradiation or, ultimately, both before and after irradiation.

The selection of thermal effect conditions on seeds, i.e., of the shock temperature and period of thermal action was based on the findings of special preliminary tests on the effect of shock temperatures on nonirradiated seeds (Nikolov, 1968; Nikolov, Ivanov, 1968). These tests proved that by keeping arabidopsis seeds in an air thermostat for 30 minutes ensures their being heated completely and evenly to the prescribed temperature; also, heating them for this period of time at temperatures over 100°C leads to reduced germinating ability and depressed root growth of sprouts produced from them. Thus a 30-minute 100°C thermal shock was always employed in the main experiments. As can be seen from Table 12, such heat treatment did not affect the germinating ability of seeds and the subsequent development of plants in the nonirradiated control group: of all the recorded features only root growth was significantly diminished (although negligibly) under the effect of thermal shocks.

The seeds were irradiated in a series of tests in a $Cs^{137}$ GUPOS unit at the Institute of Biophysics of the Academy of Sciences USSR using a 42 krad/hour dose; irradiation doses were 0, 40, 80, 120 and 160 krad. All five irradiation versions (including the nonirradiated control) were combined with all four thermal effect versions so that each experiment included 20 versions. A total of three experiments was performed with the number of test seeds per version of 120, 30 and 60, respectively; the total volume of M1 material was (120 + 30 + 60) x 20 = 4200 seeds.

TABLE 12. RECORDED FEATURES, THEIR AVERAGE VALUES IN THE NONIRRADIATED CONTROL GROUP AND THE EFFECT OF HEATING UPON THEM (100°C, 30 min.) IN TESTS ON THE INFLUENCE OF THERMAL SHOCKS ON SOMATIC AND GENETIC EFFECTS OF GAMMA IRRADIATION IN ARABIDOPSIS

| FEATURE | AVERAGE VALUE IN CONTROL | 95%-ile confidence interval | EFFECT OF THERMAL SHOCKS ON NONIRRADIATED GROUP, P |
|---|---|---|---|
| Seed rise ability, % | 91.0 | 87.3-94.1 | >0.25 |
| Survival, %: | | | |
| sprouts in cotyledon | 84.8 | 79.1-98.8 | 0.05-0.1 |
| plants in rosette ph. | 98.2 | 91.9-100 | >0.25 |
| plants in generative | 100 | -- | -- |
| Total survival rate | 83.6 | 75.9-90.1 | >0.25 |
| Growth, mm: | | | |
| main root on Day 7 of plant development | 17.0 | 16.3-17.7 | <0.001 |
| stem height on day of bloom onset | 56.1 | 53.9-58.3 | >0.25 |
| Duration of development, days: | | | |
| sowing-rosette | 5.3 | 4.5-6.1 | >0.25 |
| rosette-budding | 9.1 | 7.8-10.4 | >0.25 |
| budding-blooming | 7.8 | 7.4-8.2 | >0.25 |
| blooming-maturation | 12.8 | 11.8-13.8 | >0.25 |
| Fertility: | | | |
| number of pod seeds, ea | 16.1 | 13.5-18.7 | 0.1-0.25 |
| frequency of sterile pods, % | 30.4 | 24.3-36.9 | >0.25 |
| Mutations: | | | |
| frequency of M1 seeds splitting in chlorophyll and morphologic mutations, % | 0.53 | 0.18-1.06 | >0.25 |

The following features were taken into account in the M1: the seeds' germinating capacity, the survival rate of plants in later stages of development, the length of the main root on Day 7 of the experiment, the plants' height at the onset of the first blossom, the length of the seed-rosette-budding-blooming-maturation development stages, the number of fertile and sterile pods and the number of seeds per pod (also cf. Ch. 5).

Chlorophyll and morphologic mutations were taken stock of in M2 generations. And since one additional purpose of the test series was to obtain further data on mutations, a family record of M2 mutations was kept and all anomalies discovered were checked again in the next generation. In this respect, investigation of the M2 generation in all 20 versions would have been too labo-

35 - 36



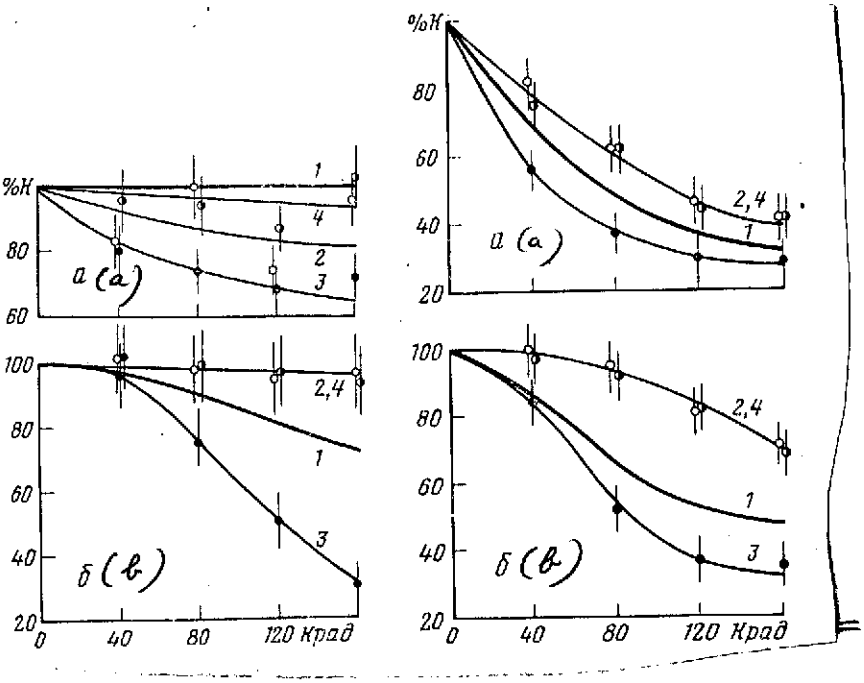



Fig. 35. Survival of sprouts in cotyledon (a) and rosette (b) phase at different gamma irradiation doses in conjunction with thermal shocks.




Fig. 36. Root growth (a) and stem growth (b) at various doses of gamma irradiation of seeds in conjunction with thermal shocks (Notations as in Fig. 35).

1) gamma without shocks; 2, 3, 4) in conjunction with shocks: preradiation (white dots), postradiation (black dots) and twofold (white/black dots).

rious. Thus complete data were obtained only for eight versions each: four without irradiation and with a 40-krad doses while plants were still rather fertile under all heat effect versions and the irradiation dose was rather large--to anticipate the occurrence of not too few a number of induced mutations.

Even with such a limitation, the total number of M2 and M3 /138 surpassed 77,000 plants.

The results obtained in the experiments were processed statistically as usual.

## 12.3. Results and Discussion of Experiments

The data obtained in the experiments showed that, as in other test series, almost all recorded features except seed germinating capacity, survival rate of plants in the generative stage and length of the bloom-maturation stage varied considerably under the effects of irradiation. Furthermore, all somatic and genetic irradiation effects, save for reduced numbers of seeds per pod,

were affected somehow or another by the effect of thermal shocks /139
and preradiation and twofold shocks as a rule showed the screening effect, while postradiation shocks increased radiation damage. Finally, in analyzing variance in data obtained between irradiation and thermal shocks, no significant interplay has been found. This enables us to express quantitatively the shock effect on the irradiation results using DCFs calculated as the mean ratio of isoeffective doses for each irradiation effect studied.

Let us no discuss in greater detail the effect of thermal shocks on different irradiation effects. The dose curves for the basic somatic irradiation effects are shown in Figs. 35-37 and the DCF values obtained using these curves, allowing for the reliability of differences obtained, are cited in Table 13. As usuall, all effects are expressed in percentages of the control group and the limits of error for this experimental point are indicated in all figures. The data on the number of mutations induced by /140
gamma irradiation of seeds (40 krad) combined with thermal shocks are given in the form of a graph in Fig. 39. The ratios of the effects at a 40 krad dose are given instead of DCF values in Table 13 on the line concerning mutations. Nevertheless, since the number of induced mutations in this dose range (cf. Fig. 23) is roughly proportional to the gamma irradiation dose, the ratios of effects under one dose should not be markedly different from the ratios of isoeffective doses.

By examining Figs. 35-39 and Table 13 together, we can see that according to most features, preradiation thermal shocks substantially reduce the radiation damage to plants (except death of sprouts in cotyledon stage, delayed vegetative development and a reduced number of seeds). Contrary to this, postradiation shocks increased radiation damage (except delayed generative development, reduced number of seeds, induced sterility and occurrence of mutations). Finally, twofold thermal shocks, like predadiation shocks, also reduce radiation damage to plants (except death of sprouts in cotyledon stage, reduced number of seeds and occurrence of mutations). It is noteworthy that the action of twofold thermal shocks in most cases was very close or even coincided with that of preradiation shocks. Thus, the /142
screening effect of preradiation shocks is not reduced by postradiative. This points to the radiation damage arising in seeds not exposed to preradiation shock and available for the modifying effect of postradiation shocks being essentially different from the unmodified postradiation shocks of damage arising in seeds exposed to preradiation shock. Without dwelling on a discussion of the possible mechanisms of shock effect, let us change directly to the question which interests us: the comparison of the effect of thermal shocks on various radiobiologic reactions in arabidopsis.

As was noted above, no significant differences are found

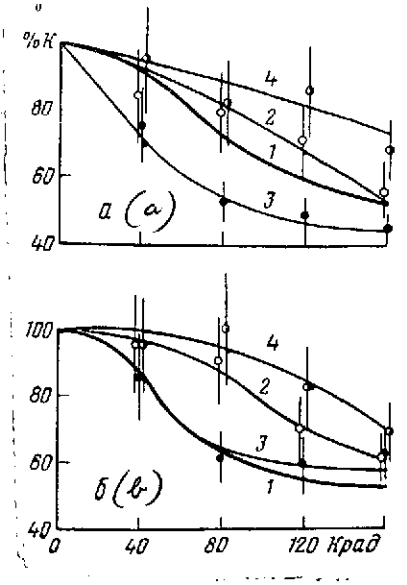



Fig. 37. Rate of vegetative (a) and generative (b) development of plants at different doses of gamma rays combined with thermal shocks.

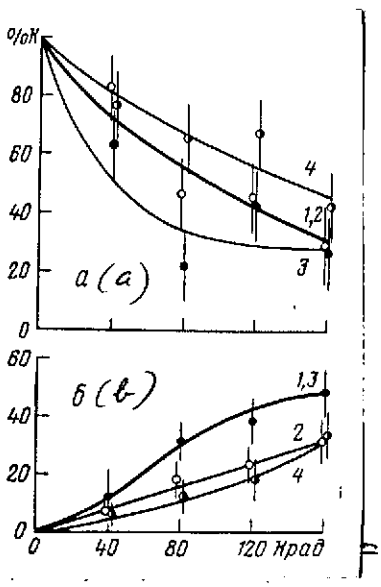



Fig. 38. Number of seeds per pod (a) and frequency of sterile pods (b) at different doses of gamma rays combined with thermal shocks (Notations as in Fig. 35).

between the dose-effect curves obtained using preradiation and twofold shocks and frequently these curves simply coincide. Therefore, the best common characteristic of the modifying effect of preradiation and twofold thermal shocks are the average DCF values according to these two factors for the irradiation effects studied. Then, using the data in Table 13, it is possible to construct the following modifiability sequence of somatic and genetic gamma-irradiation effects with preradiation and twofold thermal shocks:

$$C = N < V < M < L < S < R < G < H.$$

As for postradiation shocks, the completely different nature of their effect, as well as the absence of correlations between the irradiaton effects studied according to their modifiability by post-radiation shocks on one hand and preradiation shocks on the other hand require construction of a separate modifiability sequence for postradiation shocks:

$$G = N = S = M < H < R < L < V < C.$$

Like the analogous modifiability sequences cited in the two preceding sections, it may be thought that the mutual placement of different radiobiologic reactions in such sequences reflects the nature of their interrelationship. And again, like what has /143

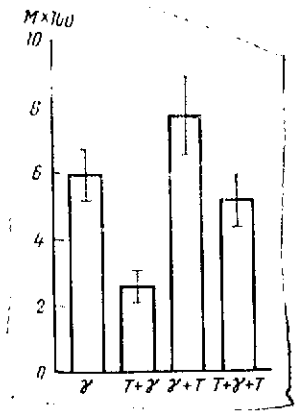



Fig. 39. Average number (M) of chlorophyll and morphologic mutations per 100 cells induced by gamma (γ) irradiation of seeds in doses of 40 krad combined with thermal shocks (T).

been stated in preceding sections, remembering the limited resources of recovering information from separate sequences on the interrelationships between different irradiation effects, we will postpone the discussion of this question to Chapter 14--all modifiability sequences will be discussed there in conjunction with each other.



TABLE 13. EFFECT OF PRERADIATION (1), POSTRADIATION (2) and TWOFOLD (3) THERMAL SHOCKS ON MAIN SOMATIC AND GENETIC EFFECTS OF GAMMA IRRADIATION IN ARABIDOPSIS

| Recorded Effect | Code | Thermal shock effect | | | | | |
|---|---|---|---|---|---|---|---|
| | | P | | | DCF | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 |
| Death of sprouts in cotyledon phase.... | C | 0.1-0.25 | <0.001 | 0.25-0.5 | 1.3 | 3.3 | 1.0 |
| Death of plants in rosette phase..... | R | 0.01-.025 | .01-.025 | .01-.025 | 0.6 | 1.4 | 0.6 |
| Depressed root growth..... | L | .001-.005 | .001-.005 | .001-.005 | 0.7 | 1.7 | 0.7 |
| Depressed stem growth | H | <0.001 | .025-.05 | <001 | 0.3 | 1.2 | 0.3 |
| Vegetative lag..... | V | .25-.5 | .001-.005 | .01-.025 | 1.0 | 2.3 | 0.7 |
| Generative lag..... | G | .005-.01 | .025-.5 | <.001 | 0.5 | 1.0 | 0.3 |
| Reduced number of seeds in pod..... | N | .9-.99 | .25-.05 | .25-0.5 | 1.0 | 1.0 | 1.0 |
| Induced sterility... | S | .025-.05 | .1-.25 | .001-.005 | 0.7 | 1.0 | 0.5 |
| Occurrence of mutations..... | M | <.001 | .25-.5 | .25-.5 | 0.4* | 1.0* | 1.0* |

*Ratio of effects at 40 krad dose.

## Chapter 13. The Effect of Neutrons of 2 MeV and 5.6 MeV on Dormant Seeds of Arabidopsis

### 13.1. General Remarks /143

As we know, a comparative study of the biologic effectiveness of inioizing radiation of various types was once one of the main experimental foundations in constructing theoretical concepts of modern quantitative radiobiology (Lea, 1946; Timofeyev-Resovskiy, Tsimmer, 1947; Buzzati-Traverso, Cavalli, 1948). In the years since then the problem of relative effectiveness of different radiations has not lost its meaning and still continues to be one of the main topics in radiobiology.

This is attributable to several circumstances. First of all, the arsenal of accessible sources of radiation is supplemented from year to year; consequently the selection of radiations used in radiobiologic research is expanding. Secondly, differences in the microgeometric distribution of absorbed energy (which distinguishes most radiation types from one another) still remain one of the most powerful instruments for studying primary mechanisms of the occurrence of radiobiologic reactions. Thirdly, another, though not new trend in radiobiology has recently begun to receive intense development: comparative study of different radiobiologic reactions by their quantitative features for the use of radiations of different types. Fourthly and last of all, the study of biologic effectiveness of radiations of different types has many practical applications.

In all these respects one of the most interesting types of emissions are neutrons. They have a very broad energy spectrum (from thermal to super-fast) and correspondingly broad spectrum /144 of LPE values. Secondly, the absence in neutrons of a charge and the great penetration capacity caused by this fact makes them suitable for the conduct of radiobiologic research on virtually any living organisms which cannot be said of many forms of heavy charged particles. Thirdly, the existence of the long period of dosimetry problems with neutrons has now been virtually overcome so that in working with them we can attain almost the same accuracy of measurement of the absorbed dose as in working with standardized X- and gamma rays. And finally, data on the biologic effectiveness of neutrons of different energies have much value in solving many applied problems such as a guarantee of personnel work safety at nuclear reactors, development of neutron capture therapy, etc.

This has all gone to define the recent appearance of many publications dealing with different aspects of study of the biologic effectiveness of neutrons of different energies, including many studies on the higher plants.

Without stopping to set forth these studies, let us note the reviews and bibliographies which contain the primary results (Boag, 1953; Zirkle, 1954; Valeva, 1960; Khvostova, Nevzgodina, 1961; Bora, 1961; Kuzin, 1964; Kondo, 1965); and also several symposiums specially dealing with biologic effects of neutrons including on the higher plants: "Biological Effects of Neutron and Proton Irradiations", IAEA, Vienna, 1964; "Neutron Irradiation of Seeds," IAEA, Vienna, 1968 and "Symposium on Neutrons in Radiobiology," US AEC Conf. 691106 (TID-4500), Oak Ridge, Tenn., 1970.

The general result of the study of different effects of neutrons on the higher plants can be formulated as follows. In irradiating dormant seeds of different species of higher plants, neutrons of different energies are much more effective than X- or gamma-rays in inducing all main radiobiologic reactions (death of plants, depression of growth and development, reduction of fertility, occurrence of different mutations and chromosomal aberration types). Thus, most values of the neutron RBE in irradiation of dormant seeds of higherplants are from 5-10 according to various authors' data; values on the order of 10-25 are often obtained; and sometimes, esepcially at small doses and low dose powers, the RBEs reach 100 or more (Neary et al., 1963; Smith et al., 1964). It is typical that in irradiating dormant seeds, the RBE of neutrons is usually higher than in irradiation of swelling seeds and sprouts (Donini et al., 1967; Fujii, 1967, 1968); for the latter, the RBE values of neutrons usually lie in the 2-5 range, which is closer to the corresponding values observed /145 in neutron irradiation of animals, especially mammals (Bora, 1958; Arsenyeva et al., 1966). Another typical feature of neutron irradiation of plants is the weak relationship of its results (or even indepencence) as functions of factors capable of greatly modifying the effects of X- or gamma-radiation (Ehrenberg, Andersson, 1954; Ehrenberg, Saeland, 1954; Smith et al., 1970). Apparently the increase in neutron RBE observed in irradiation with small doses or at very low dose powers is attributable to this fact (Neary et al., 1959, 1963).

As in other areas of plant radiobiology, in tests on neutron irradiation we usually study one or several radiobiologic plant reactions and only in recent tests has comparative study of neutron RBE in different radiobiologic plant reactions begun to attract more attention (Caldecott et al., 1954; Ehrenberg, Andersson, 1954; Ehrenberg, Saeland, 1954; Smith et al., 1964, 1968, 1969, 1970; Donini et al., 1967).

As a result of these tests it was found that the sensitivity of different plant species to neutron irradiation is, as with other types of emissions, in close relationship with the basic characteristics of the plants' nuclear apparatus--the interphase

volume of the nucleus, size and number of chromosomes, etc.(Donini et al., 1967). Therefore, the leading role of radiation damages to the nuclear apparatus in general radiosensitivity of plants is thereby confirmed.

An interesting attempt at comparative analysis of different radiobiologic reactions of plants using the neutron RBE in these reactions was undertaken by H. H. Smith and his co-authors (Smith et al., 1968). After obtaining dose curves in tests on maize for nine different final recorded effects, after irradiation of seeds with X-rays and neutrons, these authors found that between the neutron dose logarithms governing a specific yield of different radiobiologic reactions and the isoeffective doses of X-rays there exists a linear relationship. They thereby concluded that the effected that they were studying differed only in quantity and are the result of the very same mechanisms.

The arbitrary choice of effects compared is a material defect of this study. These effects were:

1) one mosaic spot on the 5th leaf; 2) two mosaic spots on the 5th leaf; 3) 50% sterile seedbuds; 4) 50% sterile pollen; 5) 50% depressed growth; 6) 50% survival; 7) 50% germination; 8) partial recovery of germinating capacity with large doses; 9) partial recovery of growth at large doses.

But both the findings obtained in this study and the numerous data of other authors prove that the shape of the dose-effect /146 curves for different features studied by them is different. Thus their linear relationship is sooner a fortunate accident caused by a successful choice of values of the compared effects than by general laws. Accordingly, it seems premature to conclude the general nature of mechanisms of occurrence of the reactions they studied. But we may state that this defect is more methodical than methodological: the authors unsuccessfully chose levels of effects for comparison while the approach itself--the comparative study of different radiobiologic reactions with the application of emissions of different types--is certainly promising.

Let us now examine data on the effect of different emissions on arabidopsis. We will begin with neutrons. We will not examine the results of our own tests with neutrons since they are set forth in detail below. The first study dealing with the effect of neutrons on arabidopsis appeared in 1961 (Daly, 1961). This study is only mentioned out of priority since it only notes that neutron irradiation of seeds induces a dose-related lag in development of plants deriving from them.

In recent years different laboratories have studied neutron RBE. The findings obtained by them are summarized in Table 14.

In irradiation of dormant seeds, RBE values obtained vary around 10, while in irradiation of swollen seeds and sprouts, they constitute 4-6. But it does not seem feasible to assess RBE variation among different reactions by the cited data.

Matters are even worse for RBE study of other forms of radiation.

According to Fujii's data, in irradiation of dormant seeds, accelerated heavy ions of $He^4$, $C^{12}$ and $Ar^{40}$ are 10, 35 and 6 times more effective, respectively than gamma-rays with respect to inducing somatic mutations (Fujii 1966; Fujii et al., 1966, 1967). Perhaps a lower RBE of $Ar^{40}$ ions is due to their poor penetrability.

According to other data (Hirono et al., 1968) obtained likewise in irradiating dormant seeds, the RBE of accelerated ions of $He^4$, $Li^7$, $C^{12}$, $O^{16}$, $Ne^{20}$ and $Ar^{40}$ are at least 10 both with respect to inducing somatic mutations and with respect to reducing the dry weight of plants. Ions of $Ne^{20}$ and $Ar^{40}$ happen to be less effective than others.

Finally, according to our preliminary data (Ivanov et al., 1968, 1968a, 1969) obtained in irradiating dormant seeds with X-rays, protons of 6.3 MeV and alpha particles of 25.1 MeV, the most effective in depressing growth, delaying development and reducing plant fertility were alpha particles, while protons were intermediate in effectiveness between alphas and X-rays.

TABLE 14. RBE OF NEUTRONS BY RESULTS OF TESTS ON ARABIDOPSIS

| Neutron features | Irradiated object | Reaction studied | RBE | Author |
|---|---|---|---|---|
| thermal neutron | dry seeds | total survival of plants | 10 | Fujii, 1968 |
| | swollen seeds | | 5 | |
| 1 MeV neutrons | 4-leaf sprouts | total survival | 4.6 | Donini et al., 1967 |
| | | dry wt. loss | 5.7 | |
| | | pollen abortiveness | 3.9 | |
| 1.5 MeV neutrons | dry seeds | somatic mutations | 16 | Fujii, 1964, 1965 |
| 14 meV neutrons | | | 13-15 | |
| fast neutrons | dry seeds | survival of cotyledon sprouts | 5-10 | Contant, Dankert 1968a; Dankert, Contant, 1968; |
| | | root growth | 5-10 | |
| | | sowing-blossoming develop't | 5-9 | |
| | | male sterility | 5-9 | |
| | | somatic mutations | 11.7 | |

At the same time, among these three types of radiation, a significant difference was not to be found with respect to inducing embryonic lethals and chlorophyll mutations. /147

In addition to analyzing RBE, in some tests on neutron irradiation of arabidopsis other questions were investigated. It was found, for example, that in irradiating seeds their sensitivity to neutrons sharply increases (Contant, 1966, 1968; Fujii, 1967, 1968), although to a lesser degree than to gamma-rays (Fujii, 1967, 1968), this upsurge being observed at the very onset of swelling (1 hour); thereafter, sensitivity of the seeds to neutrons practically does not cannge (Contant, 1968). In other tests of this same author (Contant, Dankert, 1968) no effect of postradiation storage of seed was found at -20°C for 18-53 hours on the results of their neutron irradiation. Finally, there are two studies (Contant, Dankert, 1968b, c) in which the question of correlations between different effects of neutron irardiation of seeds is examined. These studies are discussed in the next /148 chapter.

This research exhausts the literature data on the effect of different types of irradiation on arabidopsis. Of course the total picture will be complete only when we obtain data on the effect of different radiations in a broad LEL spectrum on all the chief somatic and genetic features of arabidopsis and under the most diversified test conditions. But as an initial study we decided to restrict ourselves to the study of only fast neutrons on dormant arabidopsis seeds; allowing, as in tests set forth in the preceding chapters, for all somatic and genetic effects of irradiation. The selection of fast neutrons of 2 MeV and 5.6 MeV was not accidental, since their LEL (40-50 and 30-35 keV/mc, respectively) sharply differs from the LEL of hard X-rays (no more than 2 keV/mc) and gamma-rays (no more than 0.7 keV/mc) which were used in all other tests.

The basic findings of the tests set forth here with fast neutrons are cited in the following studies (Ginter, Ivanov, 1971; Ivanov, 1969, 1970; Ivanov et al., 1970; Timofeyev-Resovskiy et al., 1971).

## 13.2. Experimental Procedures

The experiments discussed below were done using standard methods (Ch. 4-6 and 9) and on ordinary material--dormant seeds of the En-1 race of A. thaliana.

The seed irradiation neutron dose working range was determined on the basis of preliminary tests which proved that at doses of fast neutrons over 20 krad there is a 100% death of sprouts in the cotyledon phase.

In tests with neutrons of 2 MeV, irradiation of seeds was done in the fast neutron channel of a 5 MW nuclear reactor at the Physics and Power Engineering Institute in Obninsk. The main irradiation parameters were: average LEL 40-50 keV/mc, dose output about 12 krad/hr, doses 0, 4, 8, 12, 16 and 20 krad, ferrosulphate dosimetry, admixture of gamma-irradiation about 20%. A total of 4 tests were run in this series with fast neutrons with a total material volume of 60 seeds per version x 6 versions x 4 tests = 1440 test seeds of M-1. In addition, this series of tests included another 4 experiments on gamma-irradiation of seeds with a total material volume of 50 seeds per versions x 4 versions x 4 tests = 800 test seeds of M-1, with a total of 2240 seeds in the series. In the M-2 generation, the total material volume was about 105,000 embryos.

Gamma-irradiation was done on the "Luch" device of the NIIMR AMN SSSR with a cobalt source at a dose output of about 14 krad/hr, doses--0, 25, 50 and 75 krad. The need to include in the plan /149 of this test series versions of gamma-irradiation was defined by the aforenoted broad limits of variation of radiosensitivity among seed batches. Thus we had to be sure that the seed batch employed in this test series did not differ in its ssnsitivity to gamma-irradiation from the average values given in Ch. 9 and in Fig. 13-23 and adopted as the standard for evaluation of neutron RBE. Moreover, since irradiation with neutrons of 2 MeV was done with a dose output of almost 20 times less than the dose output of gamma-irradiation in most other test series, we had to be sure of the absence of the effect of the dose output of gamma irradiation, although in irradiating dormant seeds we could hardly expect this effect to be present. With a relatively low dose output of gamma rays in this test series (14 krad/hr) is related the use of relatively low doses for arabidopsis (up to 75 krad) where no lethal effect can be observed, though depressed growth and development reduced fertility and mutation occurrence are already well expressed. Data on gamma irradiation of dormant seeds obtained in this test seires are cited in Figs. 13-23, where they are denoted by the figure 10. We can see that by all features of growth, development fertility and also by the number of mutations, these data do not differ greatly from the average values in all test series. Consequently, neither noticeable deviations in radiosensitivity of seed batches nor the effect of dose output took place. The evaluation of neutron RBE by average values of effectiveness of gamma irradiation is completely justified.

In tests with Be(d,n) neutrons of 5.6 MeV, irradiation of seeds was done on a U-120 cyclotron at the Central Institute of Nuclear Research in Rossendorf near Dresden. The main parameters of irradiation were: average aalue of LEL 30-35 keV/mc, dose output about 5 krad/hr, doses 0, 5.2, 10.5, 15.6 and 20.8 krad;

dosimetry is ionization, allowing for absorption doses of the elementary composition of the seeds (Ch. 6); admixture of gamma rays about 20%. As in tests with 2 MeV neutrons and for the same reasons, this test series included irradiation of seeds by rare ionizing radiation. Only in this case, due to the lack of suitable gamma sources, the seed were subjected to hard X-ray irradiation on a TUR-M200 under the following conditions: I = 20 microamperes, V = 245 kV, filters 1 mm Cu + 2 mm Al, dose output about 6 krad/hr, doses 0, 10, 20, 30 and 40 krad. The maximum employed dose range was defined by the technical specifications of the equipment.

As we know, in irradiating dormant seeds the effectiveness of hard X-rays ordinarily does not differ from $Co^{60}$ gamma rays. This was confirmed in this test series. Data on the effect of X-ray irradiation of seeds on growth, development, fertility and mutation processes in arabidopsis are given in Figs. 13-23, /150 where they are denoted by the figure 9. We can see that by all features these data are not substantially different from the average values in all test series with gamma rays. Therefore, in this case as well the evaluation of neutron RBE by average values of gamma irradiation effectiveness is complete justified. A total of 6 tests were run in this series with neutrons of 5.6 MeV and X-rays with a total volume of material of 40 seeds per version x 9 versions x 6 tests = 2160 test seeds of M-1; in M-2 a total of about 95,000 embryos were used.

In both test series with neutrons the following features were taken into account: germination capacity of seeds, survival of plants in subsequent phases of development, root length on Day 7 after turn-up of seeds, plant height on day of onset of blossoming, length of phase germination-rosette-budding-blossoming, number of seeds per pod, frequency of sterile seedbuds, as well as the frequency of embryonic and chlorophyll mutations among embryoes of M-2.

The findings obtained were statistically processed as usual.

## 13.3. Test Findings and Discussion

Data of tests on irradiation of dormant seeds with fast neutrons of 2 MeV and 5.6 MeV proved that as a result of irradiation of seeds by neutrons of both energies, significant changes were observed in all recorded features, except for the duration of the budding-blossoming phase, while changes of features seed germination capacity and plant survival rate in the generative phase were negligible in magnitude, lying on the edge of confidence and poorly reproducible. Thus these three features are not shown in Figs. 40-44, where we cite the dose curves for the main somatic and genetic effects of irradiation. As hitherto, all effects are expressed in percentages

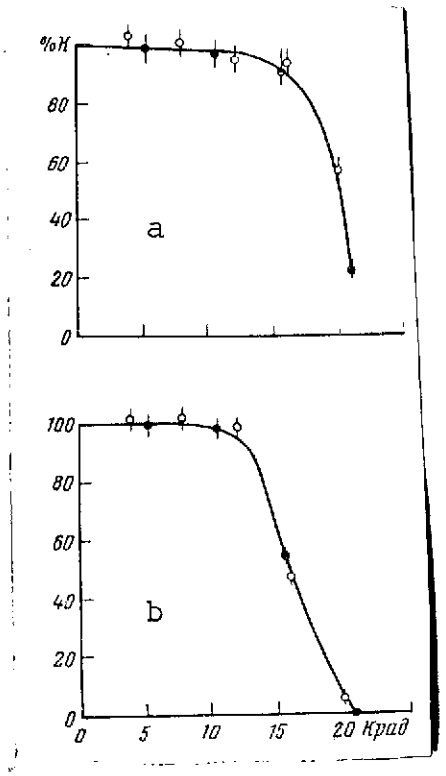



Fig. 40. Survival of sprouts in cotyledon phase (a) and rosette phase (b) at different irradiation doses of dormant seeds by neutrons: 2 MeV (white dots) and 5.6 MeV (black dots).

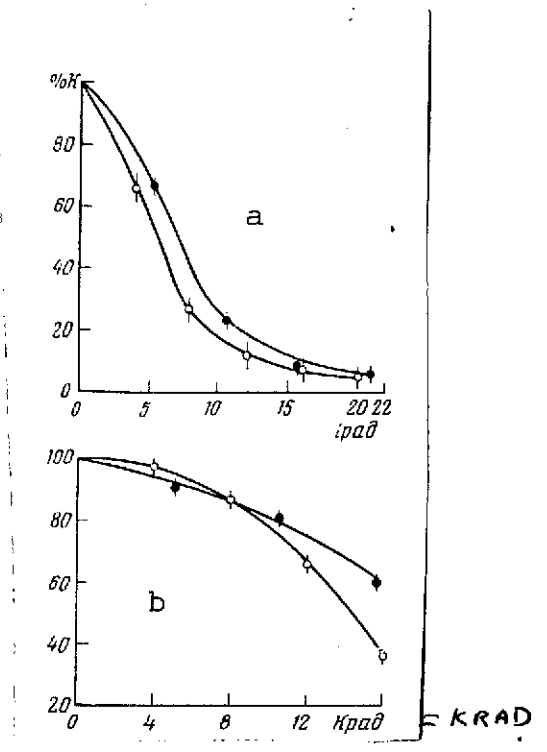



Fig. 41. Root growth (a) and stem growth (b) at different doses of irradiation of dormant seeds by neutrons (Notations as in Fig. 40).

(or minus) the control group, and all experimental points are given with their errors.

The primary attention in Figs. 40-44 is drawn by the very small variability of results--less than in tests on gamma irradiation of seeds. As we noted before (Ch. 9), the moderate variability of results is generally typical of tests on seed irradiation with neutrons; as our observations proved, this extends both to the variability of features within the experiment and to the reproducibility of findings in reiterated tests. The reason for this negligible variability in tests with neutrons is most likely the weak dependence (or independence) of the results of neutron irradiation of seeds (in contrast to their gamma irradiation) on the effect of factors concomittant to irradiation and experimental conditions (controllable and uncontrollable).

Furthermore, Figs. 40-44 show that, as in gamma irradiation/151 of seeds, as the dose of neutrons increases growth and fertili-

ty of plants is depressed; at somewhat greater doses the plants noticeably lag in development; finally plant death occurs, first in the rosettes and then in the cotyledons--all these somatic effects are combined with an increase in the number of induced mutations. In this context (with a reduction for the comparatively small number of dose points and broad limits of variability of gamma radiation findings) the shape of the dose-effect curves in neutron irradiation resembles the corresponding curves obtained in gamma irradiation of seeds (Figs. 14-23). Hence the average values of RBE of 2 MeV and 5.6 MeV neutrons for each feature were calculated in terms of RBE values at different levels of the observed effect taken with a 10% interval. Also there was no general tendency to increase nor a general tendency to decrease in the RBE as a function of the magnitude of the effects./152

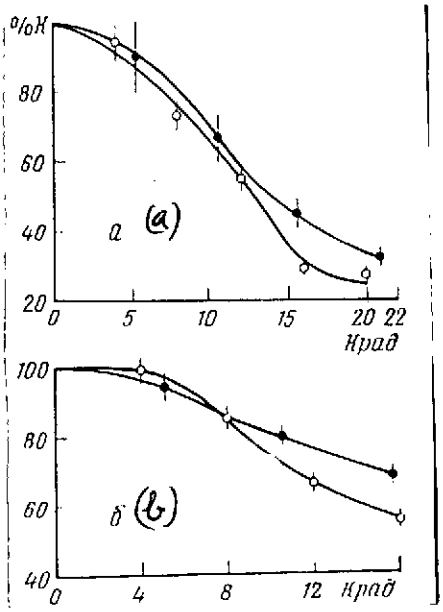



Fig. 42. Rate of vegetative (a) and generative (b) development of plants at different doses of irradiation of dormant seeds by neutrons (Notations as in Fig. 40).

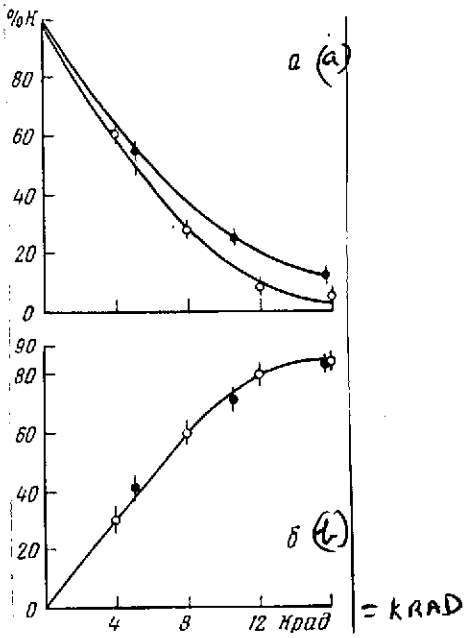



Fig. 43. Number of seeds per pod (a) and frequency of sterile seedbuds (b) at different doses of neutron seed irradiation (Notations as in Fig. 40).

Moreover, with the multiplication of the doses empl yed by the average RBE values, the confidence intervals of all experimental point obtained in both test series with neutrons intersected the corresponding averaged dose-effect curves obtained in tests on gamma irradiation of seeds. Thus, evaluation of RBE of neutrons in terms of the average ratios of isoeffective doses adequately describes their effectiveness in inducing somatic and genetic reactions as compared with gamma rays.

The RBE values of 2 MeV and 5.6 MeV neutrons obtained in this way are given in Table 15, where we can see that RBE of 2 MeV neutrons coincides with or is somewhat higher than some features than the RBE of 5.6 MeV neutrons. Great similarity in the effectiveness of neutrons of both energies can also be seen in Figs. 40-44, where the dose-effect curves for neutrons of 2 MeV and 5.6 MeV fully coincide in most features of, at least, overlap the limits of error of the experimental points. Hence, the best evaluations of RBE of particular neutrons must employ the average values in both test series. These average values of the RBE of fast neutrons in inducing somatic and genetic reactions are cited in Table 15. /153

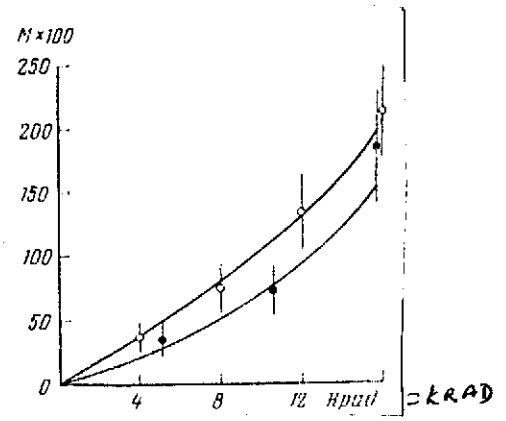



Fig. 44. Average number (M) of embryonic and chlorophyll mutations in 100 cells at different doses of irradiation of dormant seeds by neutrons. (Notations as in Fig. 40).

One methodic note on Table 15: it contains no statistical evaluations of significance of the obtained RBE values; this was done for two reasons. First of all, the effectiveness of neutrons is so much

TABLE 15. RELATIVE EFFECTIVENESS OF FAST NEUTRONS OF DIFFERENT ENERGY COMPARED TO GAMMA RAYS IN INDUCING PRIMARY SOMATIC AND GENETIC REACTIONS OF ARABIDOPSIS IN IRRADIATION OF SEEDS

| Recorded effect | Notation | RBE | | |
|---|---|---|---|---|
| | | n--2 MeV | n--5.6MeV | Avg. |
| Sprout death in cotyledon | C | 10.5 | 10.5 | 10.5 |
| Plant death in rosette | R | 8.5 | 8.5 | 8.5 |
| Delayed root growth | L | 12.2 | 9.4 | 10.8 |
| Delayed stem growth | H | 8.9 | 7.8 | 8.4 |
| Lag in vegetation | V | 11.6 | 10.4 | 11.0 |
| Lag in generation | G | 8.5 | 7.7 | 8.1 |
| Drop in number of seeds in pods | N<br>S | 13.8 | 11.4 | 12.6 |
| Induced sterility | S | 16.0 | 16.0 | 16.0 |
| Occurrence of mutations | M | 12.4 | 8.2 | 10.3 |

higher than the effectiveness of gamma rays (by one order on the average) that the extremely high statistical significance of the differences between them goes without doubt. And secondly, the path to producing final RBE values is so 'winding' and 'thorny' that it is satured with different arithmetic actions and interpolations; the RBE values are based on so many variables that the

derivation of a precise formula of RBE dispersion, if possible, /154
is extremely difficult.

Consequently, it also is extremely difficult to directly evaluate the validity of differences in neutron RBE between different features. But the obtained data permit us to make this derivation in an indirect fashion. Indeed, between the series of neutron RBE of 2 MEV and 5.6 MeV there exists a close positive correlation: $r^s = 0.71$; $P = 0.01\text{-}0.05$. The presence of this correlation between the results of completely independent tests conducted on different batches of seeds, different radiation sources (with independent dosimetry), in different years and different cities and even different countries certainly indicates the nonaccidental nature of variation in RBE within the limits of these series. Taking this fact into account, and also the proximity of effects of 2 MeV and 5.6 MeV neutrons noted before, we can construct a generalized hierarchical series of RBE values of fast neutrons in inducing somatic and genetic reactions in arabidopsis:

$$G < H < R < M < C < L < V < N < S.$$

This series together with similar series of modifiability shown in Chapters 10-12 are given here to discuss the question of interrelations between different somatic and genetic effects observed in arabidopsis after seed irradiation.

Let us make one more comment. As we know, differences in the biologic effectiveness of radiations of different types are defined by the microgeometric distribution of absorbed energy in the irradiated volume. In expressing the dose in rads, all types of emissions leave, at equal doses, an identical amount of energy per unit mass. Furthermore, both X- and gamma rays and fast neutrons of 2 and 5.6 MeV used in our tests in the final analysis transmit their energy to the irradiated substance in the same form of ionizations and perturbations of the atoms. The difference, hwoever, between X- and gamma-rays on one hand, and neutrons on the other is that ionization and perturbation induced by X- and gamma-rays are more or less uniformly distributed in the cells and only rarely accumulate as closely situated ions, especially at the end of traces, whereas ionization of atoms occurring as a result of irradiation by fast neutrons is concentrated in the form of flat columns along the tracks of nuclei of emission (mainly of protons).

Table 15 shows that even the smallest (of those obtained) average values of RBE of fast neutrons are very high (8 or more). Consequently, for the occurrence of all final recorded effects microlocal accumulations of large portions of energy leading to /155
"gross" disturbances of cell microstructure are extremely favorable.

If we take into consideration that even at the maximum dose of seed irradiation employed by us (about 300 krad) no more than 1% of all atoms are ionized (Lea, 1963), then it is possible to conclude that all recorded effects of irradiation are governed by the occurrence in each individual cell of only a very small number of "gross" microstructural disturbances.

We can scarcely imagine that a small number of injuries can even on the micro-scall of "gross" injuries be fatal for a cell if these injuries touch upon its mass components. It is incomparably more likely that these few damages are fatal only if they touch rare, unique, vitally important cell microstructures which are, as we know, genetic structures of the cell nucleus--the chromosomes. On the other hand, we we noted in Chapter 9, it is precisely fatal or, at least, stable and reproducible damages of the cells which must play the leading role in all effects of irradiation studied by us--they are (on the scale of cellular time) remote consequences of radiation effects.

Therefore, the high RBE values of neutrons in inducing somatic and genetic reactions in arabidopsis are in total agreement with other data of modern radiobiology of plants, as is discussed in the following chapter; they vie for the fact that in the occurrence of all important somatic and genetic effects observed in plants with irradiation of dormant seeds, the important role belongs to disturbances of the chromosomal apparatus of the meristemic cells, apparently, primarily to chromosomal aberrations. This does not mean that in irradiation of dormant seeds, plants cannot occur and other chromosomal damages (e.g., intragenetic changes) or different damages of other structures of the cell than chromosomes cannot occur. But their role in the quantitatively well-expressed somatic effects of irradiation, and also in the occurrence of mass types of mutations, e.g., embryonic lethals and chlorophyll mutations in arabidopsis, seems to secondary in nature.

Finally, the nonaccidental nature of differences in the effectiveness of neutrons in inducing somatic and genetic reactions in arabidopsis demonstrated above proves that radiation injuries of chromosomes are a heterogeneous group, including components of differing degrees of "grossness"; the contribution of such discrete components to the final recorded effects must be diverse.

## Chapter 14. Interrelationships between Different Effects of Irradiation in Arabidopsis

In preceding chapters, in setting forth the basic results /156
of radiobiologic tests, it was repeatedly noted that Chapter 14 would be devoted to the interrelationships between befferent radiobiologic reactions of arabidopsis. But for greater clarity of the subsequent statement, let us preceed the discussion of the question on interrelationships among different irradiation effects by a brief review of modern notions on the mechanisms of radiobiologic reactions of the higher plants.

### 14.1. Mechanisms of Radiobiologic Reactions of the Higher plants

We noted in the preceding chapter that the results of tests with fast neutrons speak in favor of the fact that in the occurrence of final recorded effects of irradiation in plants, the important role must belong to chromosomal damages. It goes without saying this conclusion must be seen only as indirect, while its value is defined mainly by the fact that it is well inscribed in the set of other data on the prevailing value of damages of the nuclear cell apparatus--more concretely--of chromosomal aberrations in the occurrence of somatic and genetic effects of irradiation in plants.

Experimental grounds of modern notions that cell radiosensitivity is defined primarily by radiation injuries to their nuclear apparatus is discussed in detail in V. I. Korogodin's book (1966). Not examining all aspects of this question, let us only mention that an important role in proving the prevailing value of radiation damages of the nuclear apparatus in general radiosensitivity of cells was played by tests on separate irradiation of the nucleus and cytoplasm (Zirkle, 1935, 1950; Petrova, 1942, 1942a; Astaurov, 1947, 1958, 1963; Strunnikov, 1960). These tests proved that with total irradiation of cells their death is observed at the same doses as in irradiation of nuclei alone; and these doses are much (sometimes by several orders) less than those at which we observe death of cells due to irradiation of cytoplasm alone.

The leading role of the nucleus in radiosensitivity of cells permits us to think that at the basis of radiobiologic reactions of multicellular higher plants there must also be radiation damages of the nuclear apparatus.

This conclusion is supported by direct proofs of the presence of a close link between radiosensitivity of plants and the main features of their nuclear apparatus (nucleus size, number and /157
size of chromosomes, DNA content, etc.). The most convincing data in this regard were obtained in a vast series of works written by A. H. Sparrow and co-authors (Evans, Sparrow, 1961;

Sparrow, Evans, 1961; Sparrow, 1962, 1965; Sparrow et al., 1961, 1965, 1967; Baetcke et al., 1967; Bowen, 1963; Miksche, 1963; Donini et al., 1967).

In these studies it was proven that with the action on seeds the sprouts or vegetative plants of varied species (about 200 species studied in toto) of acute or chronic gamma irradiation, and likewise of neutron irradiation there is a very close negative correlation, close to -1, between the size of the nucleus and chromosomes and DNA content per cell on the one hand, and miscellaneous phenomena of irradiation affection of plants (death, depressed growth, mutations) on the other. This is what should be expected, if radiation injuries of the nuclear apparatus of cells possess the main role in total plant radiosensitivity. There are some complicating circumstances in the solid overall picture obtained by the authors. For example, the correlation between nucleus size and radiosensitivity is violated if the studied group contains different ploidal species. But this does not speak against the leading role of damages to the nuclear apparatus in radiosensitivity of plants but only that in addition to the size of the nucleus for radiosensitivity its other characteristics are nowhere indifferent.

Naturally the term "radiation damages to the nuclear apparatus" is a very broad concept including also genic and chromosomal and genomic harmful mutations; but data on the correlation between nucleus size and radiosensitivity still says nothing about the relative role of each of these types of radiation damages of the nuclear apparatus in radiation affection of cells and multicellular plant organisms.

Against genic mutations as a quantitatively basic factor governing radiation affection of cells and organisms speaks the fact that the overwhelming majority of newly occurring true genic mutations are recessive and in diploid organisms their effect may only appear in subsequent generations. The occurrence in irradiation of dominant lethals and pathologic genic mutations simultaneously in many cells, which can only lead to consequences which are grave for the multicellular organism, are of extremely small likelihood.

Against genomic mutations is the fact that some of them (polyploids and hyperploids) occur in irradiation quite rarely; while others (hypoploids), although encountered relatively more often, are still not the direct result of irradiation, but occur as a consequence of chromosomal aberrations (Glotov, 1968).

Let us now consider chromosomal mutations. This type of radiation damage to the nuclear apparatus is also found in irradiation more often than others; and moreover, it is well known /158

about discrete varities of chromosomal mutations that they are either incompatible with the normal course of mitotic or meiotic division or, if these two "filters" do not form an insurmountable obstacle to them, they can appear as inherited changes in subsequent generations. Consequently, chromosomal mutations can theoretically be used as a factor of all primary final effects of irradiation observed in plant organisms.

In addition to these general concepts in favor of chromosomal mutations, experimental data also speak in favor of them as a main cause of different phenomena of radiation plant damages.

So, in tests on different plant species it was repeatedly proven the existence of a good concord between the number of divisible irradiated cells and the number of cells free of chromosomal aberrations (Gray, Scholes, 1951; Thoday, 1951; Sparrow, Christensen, 1953; Khvostova, Delone, 1959; Read, 1959; Mamedov, 1960; Davies, 1963, 1963a; Davies, Evans, 1956; Wolf, 1965). And among the number of mitotically-divisible cells on one hand and the depression of growth and development and death of plants due to radiation on the other hand, there is a direct and close association (Hall et al., 1963).

Let us mention that this concord was found also in tests on animals (Vakhrameyeva, Neyfakh, 1959; Greenblatt, 1961; Mantsygin, Bagrova, 1969) and in tests on cultures of tumor cells (Shapiro, Faleyeva, 1962; Faleyeva, Shapiro, 1964).

There are also data on the value of chromosomal aberrations in radiation-induced plant sterility. In his early studies (Gustafsson, 1940) this idea was uttered on the basis of general notions that since irradiation induces chromosomal aberrations, some of which lead to disturbance of the distribution of chromosomal material in meiosis, then chromosomal aberrations must induce sterility. In later years, this idea was confirmed by cytologic research on barley which proved that radiation-induced sterility of plants is caused mainly by tremendous chromosomal imbalances (Ehrenberg, Lundquist, 1957) and mainly by translocations (Froese-Gertzen et al., 1964). The results of tests on arabidopsis (Mueller, 1965b, 1967) also speak in favor of a genetic and not 'physiologic' nature of induced sterility. This is primarily indicated by the fact that in most cases partial sterility of plants in M1 is hereditary. Moreover, sterile seedbuds are usually distributed among pods of partially sterile M-1 plants nonuniformly, while they happen to be timed to any single chimeral sectors. It was also proven that sterility in arabidopsis is linked with the haplophase lethality of the male gametophyte. This all goes to show the genetic nature of induced /159
sterility. But problems of cytologic study of arabidopsis have not permitted us to concretize exactly which chromosomal damages

form the base of the problem.

Thus, there are direct and indirect proofs that chromosomal aberrations play a front-line role in some somatic effects of higher plant irradiation.

In a closer examination, this is valid for mutations recorded in M-2 as well; in any event for their "mass" types. As we said in Ch. 9, the assumption that among radiation-induced chlorophyll mutations in plants true genic mutations can hardly be found often and mostly comprise small deletions was first stated by D. J. Stadler (Stadler, 1932, 1954; Stadler Roman, 1948). He began on the basis that among induced chlorophyll mutations in maize dominant forms are unknown and that induced chlorophyll mutations have a strongly expressed pleiotropic effect. Both must be observed if the occurrence of chlorophyll mutations in irradiation is linked to the loss of some genetic material. Since then different authors have derived many induced chlorophyll mutations in various plants, while dominant mutations among them have thus not been found (Nilan, 1967). The pleiotropic effect of most induced chlorophyll mutations was confirmed. Meanwhile, among spontaneous chlorophyll mutations in plants, dominant forms are not encountered to often; a much smaller portion of spontaneous mutations has a strongly expressed pleiotropic effect as radiation induced (Nilan, 1967). Thus we may think that Stalder's suggestion is not void of foundations. In favor the primarily aberrant nature of radiation induced chlorophyll mutations there are also findings of Caldecott's tests with his co-authors (Caldecott et al., 1954) which found that in barely the ratio between chlorophyll mutations and chromosomal aberrations of the variable type does not change in switching from X-ray irradiation to neutron, although we can hardly expect the relative effectiveness of neutrons to be identical in inducing both genic mutations and chromosomal aberrations.

The primarily aberrant origin of not only induced chlorophyll mutations is obvious. This is indicated by the high (on the order of 10) values of RBE of neutrons regularly obtained by different authors for the most varied types of mutations (Ch. 13; Nilan, 1967; Sobels, 1969).

Finally, in individual analysed cases, the aberrant nature of induced mutations in plants has been proven cytologically. So A. Gustafsson et al. (1967) proved that among radiation induced mutations of barley harvest, cytologically recorded translocations /160 and inversions are found 15 times more often than "point" mutations.

Thus we may imagine that both somatic and genetic effects of irradiation of the higher plants are due to the occurrence of

chromosomal aberrations.

But "chromosomal aberrations" and "radiation damage to the nuclear apparatus" are collective notions, since the number of possible types of aberrations is great, and their different types have a different value both for the cell in which they appeared and for its offspring (Luchnik, 1968). It appears that even cytologically similar types of aberrations can have a different value for the fate of the cell. Thus in tests on microspores of tradescancia it was proven that although fragmentation of chromosomes accompaneid by the effect of micronuclei in the prophase of following mitosis is most often cell-lethal, but some cells with micronuclei retain viability (Koller, 1943). It is natural to think that in different final effects of plant irradiation the relative role of aberrations of different types of not identical.

Hence, between final effects of irradiation we can expect both some similarities due to their common aberrant nature and specific distinctions due to different participation of various types of aberrations in the final effects.

These similarities and differences are actually observed. Especially favorable for the exhibition of similarities and differences between final effects of irradiation is their comparative study under different experimental conditions and under the influence of different concomittant factors.

The qualitatively total sum of experiments on the comparative study of the effect of seed swelling, their postradiation storage, thermal shocks and radiation LEL (fast neutrons--gamma-rays) on the main somatic and genetic effects of irradiation observed in arabidopsis can be formulated in the following manner. Common to comparable effects of irradiation is that under the impact of these factors, all genetic and somatic effects changed in the same direction. In the event of seed swelling, their postradiation sotrage, the impact of postradiation thermal shocks and LEL increase--this was an intensification of effects; while in the event of preradiation and double thermal shocks--their attentuation. Differences between compared final effects concerned the quantitative side of matters: in the same test conditions and under the impact of the same factors, the modifiability of diverse radiobiologic reactions was heterogeneous, ranging from extremely substantial to lying on the boundary of confidence or even unreliable and poorly reproducible. But modifiability series of radiobiologic reactions under the influence of the factors studied cannot be viewed as random. In addition to qualitative comparison of the radiobiologic reactions studied, this permits us to derive a quantitative evaluation of the interrelations between them and to discuss the possible nature of such interrelations. /161

## 14.2. Evaluation of Interrelationships between diverse effects of irradiation in terms of modifiability of different factors

Let us remember that in the four preceding chapters, after citing series of modifiability of the basic somatic and genetic effects of irradiation observed in arabidopsis, we each time called attention to the fact that though in view of the broad limits of change of many effects their mutual arrangement in each individual series of modifiability should be considered inaccurate; but there is a reason to think that the structure of the modifiability series is not random. The nonrandom nature of the structure of the modifiability series is indicated by the presence of a positive correlation (sometimes very close) between series of values of the dose change factors for different features obtained either by findings of totally independent tests or, at least, between different versions of several tests.

Let us now examine all obtained series together, after writing them in a slightly different form, namely: attributing each feature with a specific rank corresponding to its place in the series of modifiability. These series are shown in Table 16. At the top part of this table are given series of modifiability of different irradiation effects obtained in terms of results of all tests, but without including the depression of stem growth (H), since in one test, namely in studying the effect of gamma rays on swelling and swollen seeds, this feature was not recorded. At the bottom of the table are given modifiability series which include all features, but without the series obtained in tests on irradiation of swelling and swollen seeds. In Table 16 we can see that the sums of ranks of modifiability of different features are somewhat differentiated, ranging from 15 (for generative development lag) to 28.5 (for sterility) and from 13.5 (for generative development lag and mutations) to 24.5 (for sprout death in cotyledon phase). But using Friedmann's criterion (Campbell, 1967, p. 54), we can be assured that these differences are not reliable at either the top of the table ($\chi^2_7 = 5.77$; $P = 0.25 - 0.5$) or the bottom ($\chi^2_8 = 4.77$; $P = 0.5-0.75$). Thus, the features examined do not differ significantly in their average modifiability.

A more detailed discussion of Table 16 proves that the compared features differ greatly in their variation with respect to /162 their averages. Thus, the number of induced mutations varied quite feebly under the impact of all factors employed (minimum rank--2.5, maximum--4). Somewhat greater, but also varying slightly, was the change of root growth (minimum rank--5, maximum --7) under the influence of these factors. All other radiobiologic reactions of arabidopsis varied slightly under the impact of several factors, were frequently most modified by others; here we can see a purely qualitative indication of the presence

TABLE 16. MODIFIABILITY OF MAIN SOMATIC AND GENETIC IRRADIATION EFFECTS UNDER THE INFLUENCE OF VARIOUS FACTORS

| FACTOR | Radiation effects compared | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | plant death | | growth depression | | development lag | | reduced fertility | | mutation occurrence, M |
| | C | R | L | H | V | G | N | S | |
| Seed swelling | 5 | 1.5 | 6 | - | 3 | 1.5 | 7 | 8 | 4 |
| Postradiation storage | 8 | 7 | 5 | - | 1 | 2 | 6 | 4 | 3 |
| Thermal shocks before irradiation and double | 1.5 | 7 | 5 | - | 3 | 8 | 1.5 | 6 | 4 |
| Thermal shock after irradiation | 8 | 5 | 6 | - | 7 | 2.5 | 2.5 | 2.5 | 2.5 |
| LEL(fast neutrons) | 4 | 2 | 5 | - | 6 | 1 | 7 | 8 | 3 |
| Total | 26.5 | 22.5 | 27 | -- | 20 | 15 | 24 | 28.5 | 16.5 |
| Postradiation storage | 9 | 8 | 6 | 4.5 | 1 | 2 | 7 | 4.5 | 3 |
| Thermal shocks before irradiation and double | 1.5 | 7 | 5 | 9 | 3 | 8 | 1.5 | 6 | 4 |
| Thermal shock after irradiation | 9 | 6 | 7 | 5 | 8 | 2.5 | 2.5 | 2.5 | 2.5 |
| LEL(fast neutrons | 5 | 3 | 6 | 2 | 7 | 1 | 8 | 9 | 4 |
| Total | 24.5 | 24 | 24 | 20.5 | 19 | 13.5 | 19 | 22 | 13.5 |

of difference degrees of similarity and difference between them.

But we can go slightly further and try to quantitatively evaluate the link between different irradiation effects in terms of their reciprocal arrangement in the modifiability series. /163 For this purpose, let us first select the measure of relative distance between the compared irradiation effects. Since the ranking distance between the i-th and j-th effects in each individual series of modifiability is $R_i - R_j$, where R is the ranks of these effects, and may be either negative or positive; and to establish links between effects in terms of the total distance between them in all n series only the distance is material and not its sign; then to avoid negative distances it is best to introduce as a measure the square of the difference of ranks $(R_i - R_j)^2$, as is ordinarily done in statistics. Then the total absolute distance between the i-th and j-th effects can be expressed as $\Sigma_n(R_i - R_j)^2$. To derive the total relative

distance between effects (D), we must subdivide the absolute distance between them by the maximally possible, i.e.,

$$D_{(i-j)} = \frac{\sum_n (R_i - R_j)^2}{n(R_{max} - R_{min})^2},$$

where $R_{min}$ = 1 and $R_{max}$ = maximally possible rank, i.e., at the top of Table 16, $R_{max}$ = 8 and at the bottom of the table $R_{max}$ = = 9.

We can see that if in all series $R_i = R_j$, then D = 0; if in all series $(R_i - R_j) = R_{max} - R_{min}$, then D - 1.

Thus a suitable expression for the coefficient of connection between two effects will be

$$K_{i \times j} = 1 - D_{(i-j)}$$

or

$$K_{i \times j} = 1 - \frac{\sum_n (R_i - R_j)^2}{n \cdot (R_{max} - R_{min})^2},$$

where

$$R_i = R_j \quad K_{i \times j} = 1,$$

and where

$$(R_i - R_j) = R_{max} - R_{min} \quad K_{i \times j} = 0.$$

After applying this coefficient of connection to the data in Table 16, we find evaluations of paired connections between all recorded final irradiation effects cited in Table 17, where the connections of stem growth depression (H) with other effects of irradiation are obtained in terms of data at the top of Table 16; others--from the bottom.

In Table 17 our attention is mainly drawn to the high overall level of connections between all final irradiation effects: only in two cases is the coefficient of connection less than 0.5 ($K_{GxC}$ = = 0.47 and $K_{GxN}$ = 0.49); in three cases it is at least 0.9 ($K_{LxM}$ = /164 = 0.90; $K_{HxR}$ = 0.93 and $K_{NxG}$ = 0.94), and the average value of the coefficient of connection is 0.76.

Furthermore, in Table 18 we can see that different final irradiation effects are connected to one another to different degrees. To better see these differences, coefficients of connection exceeding an average value of 0.76 are in boxes. We can see that in most cases (13 and 19) a close connection is ob-

TABLE 17. PAIRED CONNECTIONED AMONG BASIC IRRADIATION EFFECTS OF ARABIDOPSIS ACCORDING TO MODIFIABILITY BY DIFFERENT FACTORS

| | *M* | *S* | *N* | *G* | *V* | *H* | *L* | *R* |
|---|---|---|---|---|---|---|---|---|
| *C* | 0,74 | 0,63 | **0,81** | 0,47 | 0,75 | 0,60 | **0,89** | **0,77** |
| *R* | **0,84** | 0,61 | 0,62 | 0,86 | 0,70 | **0,93** | **0,84** | |
| *L* | **0,90** | **0,89** | **0,88** | 0,73 | **0,87** | **0,85** | | |
| *H* | **0,85** | 0,75 | 0,59 | **0,94** | 0,68 | | | |
| *V* | **0,86** | 0,73 | 0,74 | 0,70 | | | | |
| *G* | **0,89** | 0,59 | 0,49 | | | | | |
| *N* | **0,84** | **0,89** | | | | | | |
| *S* | **0,81** | | | | | | | |

TABLE 18. CORRELATIONS BETWEEN RECORDED FEATURES IN CONTROL GROUP

| | *M* | *S* | *N* | *G* | *V* | *H* | *L* | *R* |
|---|---|---|---|---|---|---|---|---|
| *C* | −0,10 | 0,04 | −0,06 | 0,31 | 0,44 | −0,40 | −0,34 | −0,11 |
| *R* | −0,09 | 0,05 | 0,43 | 0,25 | 0,06 | 0,20 | 0,44 | |
| *L* | −0,41 | −0,60 | **0,69** | −0,39 | **−0,75** | −0,16 | | |
| *H* | **0,64** | **0,66** | −0,42 | −0,03 | 0,01 | | | |
| *V* | 0,36 | 0,58 | −0,31 | **0,81** | | | | |
| *G* | 0,17 | 0,47 | 0,08 | | | | | |
| *N* | **−0,74** | **−0,65** | | | | | | |
| *S* | **0,64** | | | | | | | |

TABLE 19. CORRELATIONS BETWEEN RECORDED FEATURES WITH IRRADIATION

| | *M* | *S* | *N* | *G* | *V* | *H* | *L* | *R* |
|---|---|---|---|---|---|---|---|---|
| *C* | −0,14 | 0,00 | 0,12 | −0,58 | −0,32 | 0,39 | 0,46 | −0,05 |
| *R* | 0,02 | 0,10 | −0,34 | 0,31 | 0,36 | −0,60 | −0,31 | |
| *L* | −0,30 | −0,56 | 0,74 | −0,27 | **−0,92** | 0,23 | | |
| *H* | −0,25 | 0,04 | 0,23 | −0,60 | −0,40 | | | |
| *V* | 0,10 | 0,54 | −0,78 | 0,42 | | | | |
| *G* | −0,11 | −0,25 | 0,08 | | | | | |
| *N* | −0,55 | **−0,88** | | | | | | |
| *S* | 0,46 | | | | | | | |

served between the number of induced mutations (M) and other effects; and also between depression of root growth (L) and other effects. Only in two instances are the coefficients of connection of the number of mutations and depression of growth of root with other effects slightly below the average value ($K_{MxC}$ = 0.74 and $K_{LxG}$ = 0.73). As concerns the six other strong connections, three of them per paired connections in the group of effects R-G_h(death of plants in rosette phase--lag in generative development--depression of stem growth), and two per connections of sprout death in cotyledon phase with plant death in rosette phase ($K_{CxR}$ = = 0.77) and reduction in number of seeds ($K_{CxN}$ = 0.81) and the latter is the connection between reduction in seed number and sterility ($K_{NxS}$ = 0.89).

Therefore, between the studied final recorded irradiation effects in arabidopsis is detected a general similarity in terms of their relationship to different modifying impacts; the similarity appearing in the general high level of values of the coefficients of paired connections between them. Moreover, between these final effects we can also see a number of distinctions appearing in the distribution of strong connections.

It goes without saying, the examined system of paired connections between different radiobiologic reactions of arabidopsis, being purely phenomonologic, still says nothing about the nature of the connections observed. In adjudging this question in Chapter 10 it was indicated that we can name the minimum of three possible causes of the similarity between different radiobiologic reactions according to their modifiability, namely:

--correlativeness of compared features in plants and ontogeny;
--general nature of cellular substratum of compared radiobiologic reactions (same cells of meristemic embryo);
--similarity of mechanisms of compared radiobiologic reactions.

Let us examine these three possibilites in sequence.

Data on correlations among different recorded features in the non-irradiated control group and in irradiation of seeds are cited in Tables 18 and 19. Since among recorded features there was plant death, i.e., a feature quantitatively expressed by the fraction of dead plants in the sample, the calculated coefficients of correlation between all features in terms of the results of measurement of individual plants was impossible. Thus a ranked correlation was calculated (after Spearman) between values of compared features in reiterated experiments, namely: data of 11 experiments were used on study of postradiation seed storage. Similar computations in terms of data of 11 tests on irradiation

of swelling and swollen seeds yielded extremely similar findings. /166
The employment of a ranked correlation is connected both with the small number of specimens and with the manifest lack of correspondence in many cases (due to the very nature of the features) of the material studied to the requirement for a two-dimensional distributive normality mandatory in calculating the ordinary coefficient of correlation.

The significant parameters of rank correlation are placed in brackets in Tables 18 and 19 for clarity.

We can see that in the non-irradiated control group only a small part of the features are correlated: of 36 parameters of correlation only 8 are reliable. With irradiation, the number of correlated features drops to two pair. This is a close negative correlation between root length and the duration of vegetative development, and also between the number of seeds in pods and the frequency of sterile seedbuds among them. All other features vary independently.

In comparing parameters of correlation in Tables 18 and 19 with the coefficients of connection in Table 17, no similarity is found in their distribution by features. Therefore, the nature of interrelationships among different radiobiologic reactions of arabidopsis can not be explained by a correlation between the corresponding features in plant ontogeny.

If the similarity between different radiobiologic reactions of arabidopsis were defined by the fact that at its basis lie damages (regardless of what kind) of the same cells, we would thence expect that the most closely associated features would be within the group R - N - S - M (with the least determinacy H and G may be included in this groupping), since all of them are governed by the occurrence of injuries in cells of the reproductive part of the embryonic meristem. But the connections of features of this group with others governed by damages in cells of the vegetative part of the upper meristem of the embryo (C and V) and in cells of its rootlet meristem (L) should be much weaker.

But we can see from Table 17 that the distribution of paired connections between features is totally different: the most strongly associated with all other features were mutations and depression of root growth.

Moreover, if the force of the bond between different effects of irradiation in terms of their relationship to modifying impacts were defined primarily by the general nature of the cellular substratum of corresponding radiobiologic reactions, it would make sense to expect that in employment of any modifying actions the structure of series of modifiability would /167
remain constant. Then, with a deduction for inaccuracy of

TABLE 20. CORRELATIONS BETWEEN DIFFERENT FACTORS IN TERMS OF THEIR MODIFYING EFFECT ON THE MAIN EFFECTS OF IRRADIATION IN ARABIDOPSIS

| Factors | LEL (fast neutrons) | Postradiation thermal shocks | Preradiation & double thermal shocks | Postradiation seed storage |
|---|---|---|---|---|
| Swelling of seeds | 0.85 | -0.08 | -0.43 | 0.26 |
| Postradiation seed storage | 0.11 | 0.34 | -0.27 | |
| Preradiation and double thermal shocks | -0.62 | -0.23 | | |
| Postradiation shocks | 0.06 | | | |

of the mutual arrangement of members of each modifiability series constructed according to experimental data, we should expect that among all series we will at least observe a positive correlation. In reality, things are not so. As seen from Table 20, a significant positive correlation is observed only between swelling and LEL, while between these factors on one hand and preradiation and double thermal shocks on the other we may sooner speak of a negative correlation. The action of all other factors on the final effects of irradiation is not correlated.

In this manner, the assumption that the basis of similarities among different final effects (with respect to their modifiability) is formed by the general nature of cellular substratum of the corresponding radiobiologic reactions also does not agree with the actual data.

A third possibility remains that the similarities and differences among final effects with respect to their modifiability by effects utilized reflect the corresponding similarities and differences in the mechanisms inducing the occurrence of these final effects.

We cannot, of course, affirm that both ontogenetic correlations and the general nature of the cellular substratum of reactions have no significance in the appearance of similarities and differences among recorded final effects of irradiation. We may only imagine that their relative function is less than the function of similarities and differences in the mechanisms of occurrence of the effects.

Until now we have based the comparisons on paired coefficients of connection between the irradiation effects studied. But,

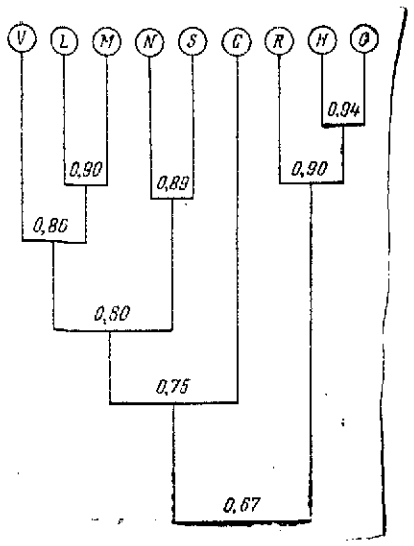



Fig. 45. Interrelationships between different irradiation effects observed in arabidopsis. (Explanations in the text).

if we use methods of numerical /168 taxonomy (Bailey, 1970, p. 148) we can also construct a general system of associations among them.

This system of interrelationships between the main somatic and genetic effects of irradiation observed in arabidopsis is shown in Fig. 45. At the top of the figure are given the arbitrary notations of nine radiobiologic reactions of arabidopsis (legend given in Table 16; horizontal lines show the levels of associations among different reactions and their groups).

We can see in Fig. 45 that in a general system of interrelationships three groups of most closely associated irradiation effects are emphasized: V - - L - M, N - S and R - H - G, while death of sprouts in the cotyledon phase (C) does not enter into a single such group. Attention is called to the fact that even the smallest of the intergroup connections constitutes 0.67. This high value of this coefficient of connection once again underscores the theoretical generality of the initial mechanisms of all final irradiation effects, both somatic and genetic. As was proven in the previous section of this chapter, there are grounds to consider that on the cellular level the occurrence of chromosomal aberrations is such a general and basic mechanism of radiation damage of plants.

Consequently we can imagine that the division of the entire set of effects into several groups describes the dissimilar relative contribution of chromosomal aberrations of different types to different final effects of plant irradiation and, conversely, that the tight link between several effects within the groups indicates that the leading role belongs to the same type aberrations in their occurrence.

Examining the composition of groups in Fig. 45 from this viewpoint, we may utter several ideas on the nature of the intragroup associations.

Let us first examine the group V - L - M. Based on what has been said, we may imagine that the basis of delay in vegetative plant development, depression of root growth and occurrence of embryonic and chlorophyllic recessive lethals are identical or,

at least, extremely similar injuries on the cellular level. Which /169
of these three final effects will be recorded depends on the cells in which these damages occur: for the appearance of mutations in M-2 it is necessary that changes in the chromosomes occur in cells of the reproductive part of the upper meristem; for delay in vegetative development--in cells of the vegetative part of this meristem; and for depression of growth of roots--in cells of the root meristem. In the latter two cases we can scarcely think of a material effect of damages in cells of one of the embryonic meristems on another, since both depression of root growth and delay in vegetative development of plants are taken into account in this growth, where the viability of sprouts is ensured still by the nutrition reserves of the seed. Undoubtedly, radiation damages to the chromosomes really occur in the upper and root meristem, so that all these three final effects of irradiation can be observed simultaneously. Remarkably, depression of both root growth and vegetative development at 50% of the maximally possible for these features is observed at the same doses of gamma irradiation of dormant seeds (50-70 krad) at which the average number of mutations per 100 cells reaches 50 (Fig. 13, 17b and 23b). This makes us think that among meristemic cells of embryos of dormant seeds there are no serious distinctions in radiosensitivity, and the degree of depression of root growth and vegetative development is roughly proportional to the number of damaged cells.

Let us now examine the group G - H - R. In contrast to the preceding group (V - L - M), all final effects entering into this group are more or less associated with damages of the came cells of the reproductive part of the apical embryo meristem. The delay in vegetative development and subsequent depression of stem growth are observed at relatively weak doses of irradiation; at higher doses, generative development of plants becomes impossible and they die in the rosette phase. Consequently, we can think that the first two features (indeed, linked with each other more than the others) are only different final effects of the same damages; and the third--death of plants in the rosette phase--differs from them only in quantity: its appearance requires a large number of damages.

The close connection of features N and S is scarcely worth a special discussion, since it is obvious: the main cause of a reduction in the number of seeds is the radiation-induced sterility. The inclusion of both these features, and not just one of them served as a type of control, to once again be sure that the study of interrelationships among different irradiation effects by their modifiability is grounded in reality. Indeed, in Chapters 10-13 we might have seen that the similarity among these features was not always so great as would have been expected. Often one or the other exhibited more similarity with some other /170
feature than with its analogue. Nonetheless, when all data obtained were analyzed together, the natural connection between the

number of seeds and sterility exceeded all connections of these features with others.

Finally, let us now examine the death of sprouts in the cotyledon phase (C). This feature does not exhibit a close connection with a single group examined. Thus we can think that in addition to the accumulation of radiation damages to chromosomes in the cells which are responsible for all other final irradiation effects, a perceptible contribution to cotyledon death may be also made by damages to other structures and cell components--even more so since this form of death is only observed at the highest doses of irradiation. Therefore, if it follows from all data of modern radiobiology that the basis of all somatic and genetic effects of irradiation of the higher plants is damage to the chromosomes, then the analysis of interrelationships among these final effects in terms of their different modifying effects allows us to affirm that the relative contribution of different types of damage to the chromosomes in different final effects is dissimilar.

Naturally this conclusion would sound more convincing if we could reinforce it with direct experimental data. But further progress in this direction in tests on arabidopsis depends more on the successes of cytogenetics of this plant than on radiobiology and radiation genetics. The successes of cytogenetics, and cytology of arabidopsis are still extremely meager.

This is also valid with respect to the conclusion that all basic somatic and genetic irradiation effects of plants are governed primarily by radiation damages to the nuclear cell apparatus, and more concretely--by different types of chromosomal aberrations.

# CONCLUSION /171

Only a few years have passed since the hard-to-pronounce word Arabidopsis began regularly appearing on the pages of biology journals and formed the heading of a book (Redei, 1969). But in that short time, this small little plant has succeeded in recommending itself in the most varied fields of modern biology--from molecular bases to geographic aspects. And radiobiology and radiation genetics have not been by-standers. An attempt was made on the pages of this book to generalize the findings of all radiobiologic and radiation genetic studies of arabidopsis.

What then is the final result of tests on irradiation of arabidopsis?

First of all, earlier proposed important methodic merits of arabidopsis as an object of radiobiologic and radiation genetic research, primarily those of general notions, were confirmed. Such merits include the small size of the plants, the short life cycle, the high seed productivity, the possibility of rapid mass sowing of plants in small areas under homogeneous and controlled laboratory conditions, and the presence of many somatic and genetic features suitable for quantitative analysis.

In a short period of time this has all allowed us to produce a rather complete radiobiologic and radiation genetic description of arabidopsis, including data on the main somatic and genetic effects of ionizing radiations of diverse types and on the effect of modifying impacts on these phenomena.

Particularly valuable was a comparison of the main somatic and genetic effects of arabidopsis irradiation under different radiobiologic test conditions. This comparison permitted the concretization of the idea of the leading role of chromosomal aberrations in radiation injury of plants, proving that the relative input of chromosomal aberrations of different types to final irradiation effects must be dissimilar. This conclusion, /172 of course, still must be made but having direct cytologic data on the comparative modifiability of different types of radiation-induced chromosomal aberrations is yet to come. But that is a matter for cytologists and cytogeneticists. And if cytologists and cytogeneticists working with arabidopsis will not be presently able to surmount their methodic problems, they will thereby risk losing the crown of laurels to their colleagues working with other long and well known objects.

But no matter what cytologists and cytogeneticists say, the phenomenon of modifiability of different final irradiation effects by the same modifying impacts, as well as the existence of specific interrelationships among different final effects are interesting per se.

They are primarily interesting in theoretical respects, since they permit us to deepen existing ideas on the mechanisms of radiobiologic and radiation genetic reactions and to give demonstration, from new positions, of the material function of radiation damages to the genetic apparatus of cells in all main final phenomena of radiation injury to the multicellular organisms of plants.

Findings obtained in radiobiologic tests on arabidopsis are also valuable in applied respects.

Thus the fact that even such nonspecific factors as swelling of seeds, their postradiation storage, thermal shocks and radiation LEL dissimilarly modify different final effects of irradiation permits us to hope for success in searching for methods of selective action on radiation damage to plants--by intensifying some effects, we can weaken others or leave some untouched. Apparently the most promising is the search among chemical agents which differ in greater specificity of effect than the named physical factors. The value of selective effect on different radiobiologic reactions, e.g., for practical radiobiologic selection of plants, is quite obvious.

On the other hand, the same distinctive effect of these nonspecific factors on irradiation effects indicates the advisability of seeking universal means of influencing all phenomena of radiation plant injury. This conclusion would seem to be even more valid with respects to multicellular animal organisms, incomparably more complex and differing in greater specialization of structures and functions than the higher plants. This means that in the search for antiradiation drugs, there are many fewer changes to rely on cardinal and universal efficiency of one single substance than on the activity of a combination of substances, each of which most efficiently acts /173
with respect to some single radiobiologic reaction or group of interrelated reactions.

And more. The fact that in irradiation of dormant seeds their radiation affection is defined by events connected with the microlocal absorption of large portions of energy makes us think that it is hardly necessary to count on the possibility of a very strong effect on the primary mechanisms of radiobiologic reactions of plants of any concomittant factors, since these factors, be they physical or chemical, are energy-wise comparable with microlocal accumulations of a large number of ionizations. It is different if the effect of factors employed is directed at postradiation processes in injured cells or affects the fate of uninjured meristem cells. Then we can expect effects of any order. Is this not because some of the most active protectors in plant irradiation are the growth substances and hormones activating cellular division? It appears that the microlocal absorption of large portions of en-

ergy has a relatively lesser value in irradiation of cells in the state of active viability. This is proven by smaller values of RBE than in irradiating dormant plant seeds of emissions with high LEL obtained in tests on sweollen seeds and vegetating plants, and also in tests on animals. Consequently, we can expect that primary injuries in cells in the state of active viability must be more accessible for modifying impacts if, of course, such state does not excessively accelerate the conversion of incipient injuries into an irreversible form. If the latter is valid, then in irradiation of cells in a state of active viability it's more possible to "help" uninjured tissues, organs or organssms than to "save" hopelessly injured cells. Is this not proven by the fact that substitutive bone marrow therapy still remains the most effective, more effective than any of the now thousands of tested drugs? Certainly all these questions merit fixed attention and further study.

Neither in this study nor in world radiobiologic literature on arabidopsis are there yet reflections of many vital questions of radiobiology and radiation genetics.

For example, such problems as postradiation recuperation, chemical protection, the effect of small doses of radiation and many others.

The study of radiobiology and radiation genetics of arabidopsis is certainly interesting as far as employing this plant as an object of research on aerospace biology. First of /174
all, in the course of radiobiologic and genetic research the doubtless merits of arabidopsis as one of the promising objects of aerospace biology have been manifested. Secondly, in the course of radiobiologic and genetic research methods of experimental work with arabidopsis have been developed and improved. And finally, findings of radiobiologic and genetic research of arabidopsis under ground-based conditions have lain the necessary ground-work without which the study of radiation effects on this plant under space flight conditions would be impossible.

It remains merely to hope that the merits of arabidopsis will as soon as possible be evaluated by biologists working on aerospace research and that this plant will have the red carpet lain into space.

BIBLIOGRAPHY


1. Abdulayev, Kh. A., Tageyeva, S. V., Kasyanenko, A. G., Usmanov, P. D., Nasyrov, Yu. S., Dokl. AN TadzhSSR, 13, 47 (1970. /175
2. Abdulayev, Kh. A., Usmanov, P. D. Tageyeva, S. V., Nasyrov, Yu. S., Geneticheskiye aspekty fotosinteza [Genetic aspects of photosynthesis], Dushanbe, 1971, p. 77.
3. Abdulayev, Kh. A., Usmanov, P. D., Tageyeva, S. V., Nasyrov, Yu. S., Arabidopsis Information Service [AIS] 9, 26 (1972).
4. Al'shits, L. K., "The connection between changes in pea seed radiosensitivity in the process of swelling and sprouting and postradiation recuperation," Cand. diss., Sverdlovsk, 1969.
5. Arsenyeva, M.A., Golovkina, A. V., Belyayeva, L. A., Vliyaniye ioniziruyushchikh izlucheniy na nasledstvennost' [The effect of ionizing emissions on inheritance], Moscow, 1966, p. 115.
6. Astaurov, B. L., Zhurn. obshch. biol., 8, 421 (1947).
7. Astaurov, B. L., Byull. MOIP, otd. biol., 63, 55 (1958).
8. Astaurov, B. L., Pervichnyye mekhanizmy biologicheskogo deystviya ioniziruyushchikh izlucheniy [Primary mechanisms of biologic action of ionizing radiations], Moscow, 1963, p. 140.
9. Atayan, R. R., "The effect of temperature shocks and oxygen on radiation damages to chromosomes", Cand. diss. Echmiadzin, 1968.
10. Afanasyeva, A. S., Byull. MOIP, otd. biol. 45, 433 (1936).
11. Batalov, R. B., Kvitko, K. V., Fotosintez i ispol'zovaniye solnechnoy energi [Photosynthesis and the use of solar energy], Nuachn. sovet po probleme "Fotosintez" AN SSSR, Inst. fiziol. i biofiz. rast. AN TadzhSSR, Dushanbe, 1971, p. 257.
12. Batalov, R. B., Kvitko, K. V., Geneticheskiye aspekty fotosinteza [Genetic aspects of photosynthesis], Dushanbe, 1971a, p. 44.
13. Batalov, R. B., Kvitko, K. V., Usmanov, P. D., AIS 9, 25 (1972).
14. Berezina, N. M., Predposevnoye oblucheniye sel'skokhozyaystvennykh rasteniy [Presowing irradiation of agricultural plants] Moscow, 1964.
15. Berezina, N. M., Merkulov, A. S., Norbayev, N., Radiotoksiny, ikh priroda i rol' v biologicheskom deystvii radiatsii vysokikh energiy [Radiotoxins, their nature and role in the action of radiation of high energies], Moscow, 1966, p. 91.
16. Bochantsev, V. P. Bot. materialy gerbariya Bot. inst. im. V. L. Komarova, 18, 104 (1957).
17. Bochantsev, V. P., Bot. materialy gerbariya Bot. inst. im. V. L. Komarova 19, 105 (1959).

18. Bochantsev, V. P., Novosti sistematiki vysshikh rasteniy [News in systematics of the higher plants], Leningrad, 1965, p. 268.
19. Bochantsev, V. P., Vvedenskiy, A. I., Flora Uzbekistana [Flora of Uzbekistan], Part 3, 1955, Tashkent.
20. Breslavets, L. P. Rasteniye i luchi Rentgena [The plant and X-Rays], Moscow, Leningrad, 1946.
21. Bush, N. A., Kriticheskaya flora Kavkaza 3, 4 (1909).
22. Bush, N. A., Flora SSSR [Flora of the USSR], Moscow-Leningrad, 1939, 8, p. 76.
23. Valeva, S. A., Biofizika 5, 362 (1960).
24. Vasilyeva, A. N., Flora Kazakhskoy SSR 4 (1961).
25. Vasil'chenko, I. T., Flora Turkmenii 3 (1948).
26. Vakhrameyeva, N. A., Neyfakh, A. A. Dokl. AN SSSR 128, 429 (1959).
27. Velikanov, L. P., Pumpyanskaya, S. L., AIS 7, 36 (1970). /176
28. Giller, Yu. Ye., Asoyeva, L. M., Kasyanenko, A. G., Geneticheskiye aspekty fotosinteza [Genetic aspects of photosynthesis], Dushanbe, 1971, p. 107.
29. Giller, Yu. Ye., Kasyanenko, A. G., Vakhidova, L. R., Yusupova, G. A., Fotosintez i ispol'zovaniye solnechnoy energii, [Photosynthesis and the use of solar energy] Dushanbe, 1971, p. 247.
30. Ginter, T. N., Ivanov, V. I., AIS 5, 23 (1968).
31. Ginter, T. N., Ivanov, V. I., AIS 8, 29(1971).
32. Glotov, N. V., Genetika 4, 24 (1968).
33. Grigoryev, Yu. S., Opredelitel' rasteniy okrestnostey Stalinabada [Guide to Plants in the suburbs of Stalina bad], Moscow-Leningrad, 1953.
34. Dishler, V. Ya., Genetika 4, 124 (1965).
35. Zakirov, V. Z., Flora i rastitel'nost' basseyna reki Zeravshan [Flora and vegetation of the Zeravshan River Basin], Tashkent, 1961, part 2.
36. Zyablitskaya, Ye. Ya., AIS 6, 3 (1969).
37. Zyablitskaya, Ye. Ya., Bot. zhurn. 57, 331 (1972).
38. Ivanov, V. I., AIS 4, 58 (1967).
39. Ivanov, V. I., AIS 6, 26 (1969).
40. Ivanov, V. I., Materialy I Vsesoyuzn. simp. po radiobiol. rast. organizma [Materials of the First All-Union Symposium on Radiobiology of Plant Organisms], Kiyev, 1970, p. 15.
41. Ivanov, V. I., AIS 8, 29 (1971).
42. Ivanov, V. I., Ginter, Ye. K., Glotov, N. V., Radiatsiya i organizm [Radiation and the Body], Obninsk, 1970.
43. Ivanov, V. I., Ginter, T. N., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., Timofeyev-Resovskiy, N. V., Khaberer, G., Studia biophysica 27, 131 (1971).
44. Ivanov, V. I., Zyablitskaya, Ye. Ya., Sanina, A. V., Usmanov, P. D., Khaberer, G., Studia biophysica 13, 111 (1969).
45. Ivanov, V. I., Ivanova, T. A., Sanina, A. V., Smirnova,

T. N., Timofeyeva-Resovskaya, Ye. A., Radiatsiya i organizm. Mat. yubil. konf. IMR AMN SSSR, Obninsk, 1967.
46. Ivanov, V. I., Kasyanenko, A. G., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., Genetika 8, 55 (1966).
47. Ivanova, V. I., Sanina, A. V., AIS 4, 56 (1967).
48. Ivanov, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A. Radiobiologiya 8, 118 (1968).
49. Ivanov, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., Genetika, 5, 16 (1967).
50. Ivanova, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., AIS 5, 48 (1968a).
51. Ivanova, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., Mekhanizmy biologicheskogo deystviya ioniziruyushchikh izlucheniy. Tezisy dokl. II Respubl. konf. [Mechanisms of biologic action of ionizing radiations. Summaries of reports of the II Republican Conference], Lvov, 1969, p. 122.
52. Ivanov, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., AIS 6, 25 (1969a).
53. Ivanov, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., AIS 6, 25 (1969b).
54. Ivanov, V. I., Sanina, A. V., Timofeyeva-Resovskaya, Ye. A., Khaberer, G. Studia biophysica 10, 149 (1968). /177
55. Ivanov, V. I., Timofeyeva-Resovskaya, Ye. A., AIS 3, 33 (1966).
56. Ikonnikov, S. S., Opredelitel' rasteniy Namira [Guide to plants of the Namir], Dushanbe, 1963.
57. Kasyanenko, A. G., Tezisy dokl. II nauchn. konf. Tadzh. biokhim. obshchestva [Summaries of reports of the II scientific conference of the Tadzhik biochemical society], Dushanbe, 1964.
58. Kasyanenko, A. G., Nekotoryye voprosy radiobiologii[Some problems in radiobiology], Dushanbe, 1964a, p. 41.
59. Kasyanenko, A. G., Tezisy Vsesoyuzn. simp. po eksperim. mutagenezu zhivotnykh, rasteniy i mikroorganizmov [Summaries of reports of the All-Union symposium on exp. mutagenesis of animals, plants and microorganisms], Moscow, 1965.
60. Kasyanenko, A. G., "The effect of genetic factors on the functional features of the photosynthetic apparatus," Cand. diss., Dushanbe, 1966.
61. Kasyanenko, A. G., AIS 6, 29 (1969).
62. Kasyanenko, A. G., Fotosintez i ispol'zovaniye solnechnoy energii, Dushanbe, 1971, p. 244.
63. Kasyanenko, A. G., Giller, Yu. Ye., Genetika 3, 42 (1967).
64. Kasyanenko, A. G., Loginov, M. A., Nasyrov, Yu. S., In: Geneticheskiye aspekty fotosinteza, Dushanbe, 1971, p. 120.
65. Kasyanenko, A. G., Nasyrov, Yu. S., Fiziol. rast. 15, 422 (1968).

66. Kasyanenko, A. G., Nasyrov, Yu. S., AIS 5, 26 (1968a).
67. Kasyanenko, A. G., Nasyrov, Yu. S., AIS 7, 31 (1970).
68. Kasyanenko, A. G., Nasyrov, Yu. S., Smolina E. A., In: Geneticheskiye aspekty fotosinteza, Dushanbe, 1971, p. 56.
69. Kasyanenko, A. G., Timofeyev-Resovskiy, N. V., Byull. MOIP, otd. biol. 72, 100 (1967).
70. Kasyanenko, A. G., Usmanov, P. D., AIS 6, 30 (1969).
71. Kvitko, K. V., Vestnik LGU, ser. biol. 15, 47 (1960).
72. Kvitko, K. V., Mueller, A., In: Issledovaniya po genetike [Research in genetics], Leningrad, 1961, 1, p. 79.
73. Korogodin, V. I., Problemy postradiatsionnogo vosstanovleniya [Problems of post radiation recuperation], Moscow, 1966.
74. Kuzin, A. M., Radiobiologiya 4, 18 (1964).
75. Kulik, M. I., Dokl. AN SSSR 162, 928 (1965).
76. Lebedeva, L. I., Yermolayev, V. K., Kerkis, Yu. Ya., Dokl. AN SSSR, 169, 1449 (1966).
77. Loginov, M. A., Usmanov, P. D., AIS 9, 5 (1972).
78. Luchnik, N. V., Biofizika tsitogeneticheskikh porazheniy i geneticheskiy kod [Biophysics of cytogenetic affections and genetic code], Leningrad, 1968.
79. Mamedov, T. G., Tsitologiya 2, 175 (1960).
80. Mantsygin, Yu. A., Bagrova, A. M., Radiobiologiya 9, 875 (1969).
81. Nasyrov, Yu. S., Kasyanenko, A. G., Dokl. AN TadzhSSR 8, 39 (1965).
82. Nikitina, Ye. V., In: Flora Kirgizskoy SSR, 1955, p. 6.
83. Nikolov, G. V., "The effect of thermal shocks on radiobiologic and radiation-genetic reactions of Arabidopsis thaliana (L.) Heynh. with gamma irradiation of dormant seeds," Cand. diss. Moscow University, 1968.
84. Nikolov, Ch. V., Ivanov, V. I., Radiobiologiya 8, 442 (1968).
85. Nikolov, Ch. V., Ivanov, V. I., Genetika 5, 166 (1969).
86. Nuzhdin, I. N., Dozortseva, R. L., Samokhvalova, N. S., Zh. obshch. biol. 24, 261 (1963).
87. Nuzhdin, N. I., Filev, K. A., Dokl. AN SSSR 160, 224 (1965).
88. Pasyaukova, V. V., In: Ushchelye Kondara [Kondar Gorge], Dushanbe, 1951.
89. Polyakova, T. F., In: Issledovaniya po genetike [Research in genetics], Leningrad University, 1964.
90. Pomogaybo, V. M., Tsitologiya i genetika 2, 507 (1968).
91. Pomogaybo, V. M., Tsiologiya i genetika 3, 243 (1969). /178
92. Poryadkova, N. A., Timofeyev-Resovskiy, N. V., Luchnik, N. V., Tr. In-ta biol. UFAN SSSR [Proceedings of the Institute of Biology of the Department of Flora of the Academy of Sciences USSR] 12, 159 (1960).
93. Preobrazhenskaya, Ye. I., Timofeyev-Resovskiy, N. V.,

Dokl. AN SSSR, 143 (1962).
94. Rapoport, I. A., Dokl. AN SSSR 59, 1183 (1948).
95. Rapoport, I. A., Ed., Supermutageny [Supermutagens], Moscow, 1966.
96. Savin, V. N., Stepanenko, O. G., Stoleshnikova, I. V., AIS 7, 37 (1970).
97. Sanina, A. V., "Genetic and somatic effects of gamma-irradiation in Arabidopsis thaliana (L.) Heynh and their modifications in storage of irradateed seeds," Cand. diss. Obninsk, 1970.
98. Sanina, A. V., Ivanov, V. I., Timofeyeva-Resovskaya, Ye. A., Radiobiologiya 10, 629 (1970).
99. Semerdzhyan, S. P., Oganesyan, D. O., Petrosyan, L. A., Nauch. tr. Inst. zemledeliya ArmSSR, 1968, p. 363.
100. Serebrovskiy, A. S., Geneticheskiy analiz [Genetic analysis] Moscow, 1970.
101. Strunnikov, V. A., Tsitologiya 2, 573 (1960).
102. Takhtadzhyan, A. L., Sistema i filogeniya tsvetkovykh rasteniy [System and phylogeny of flowering plants] Moscow-Leningrad, 1968, p. 208.
103. Timofeyev-Resovskiy, N. V., Ginter, Ye. K., Glotov, N. V., Ivanov, V. I., Genetika 7, 42 (1971).
104. Timofeyev-Resovskiy, N. V., Ivanov, V. I., Korogodin, V. I., Primeneniye printsipa popadaniya v radiobiologii [Use of the principle of incidence in radiobiology], Moscow, 1968.
105. Timofeyev-Resovskiy, N. V., Tsimmer, K. G., Biophysik 1 (1947), Leipzig.
106. Timofeyeva-Resovskaya, Ye. A., Timofeyev-Resovskiy, N. V., AIS 4, 57 (1967).
107. Titova, N. N., Sov. botanika 2, 61 (1935).
108. Urbakh, V. Yu., Biometricheskiye metody [Biometric methods] Moscow, 1964.
109. Usmanov, P. D., Genetika 4, 105 (1966).
110. Usmanov, P. D., AIS 7, 32 (1970).
111. Usmanov, P. D., In: Fotosintez i ispol'zovaniye solnechnoy energii, Dushanbe, 1971, p. 241.
112. Usmanov, P. D., Loginov, M. A., Izrafilova, U., Akhmedov, A. Ya., Yunusov, S. Yu., AIS 7, 34 (1970).
113. Usmanov, P. D., Mueller, A., Genetika 6, 50 (1970).
114. Faleyeva, Z. N., Shapiro, I. M., Dokl. AN SSSR 159, 1158 (1964).
115. Fershtat, A.M., Savin, V.N., Stepanenko, O.G., AIS 8, 3 (1971).
116. Khvostova, V.V., Delone, N. L., Tsitologiya 1, 320 (1959).
117. Khvostova, V.V., Nevzgodina, L.V., Radiobiologiya 1, 611 (1961).
118. Khvostova, V.V., Nevzgodina, L.V., In: Raditsionnaya genetika [Radiation genetics] , Moscow, 1962, p. 358.
119. Khvostova, V.V., Nevzgodina, L. V., Dubinin, N. P. Dokl.

AN SSSR, 161, 1219 (1965).
120. Shapiro, I.M., Faleyeva, Z.N., Radiobiologiya 2, 749 (1962).
121. Shapiro, N. I., Protopopova, Ye. M., Radiobiologiya 4, 270 (1964).
122. Shkvarnikov, P.K., Chernyy, I.V., Radiobiologiya 4, 297 (1964).
123. Eydus, L. Kh, Ganassi, Ye. E., Biofizika 5, 523 (1960).
124. Yunusov, S. Yu., Kasyanenko, A.G., Usmanov, P. D., Izv. AN TadzhSSR 35, 3 (1969).

Foreign Authors

125. Abdalla, F.H., Roberts, E. H., Ann. Bot. 33, 153 (1969).
126. Abeels, M., AIS 5, 27 (1968).
127. Abeels, M., Digneffe, C., AIS 6, 11 (1969).
128. Abeels, M., Digneffe, C., Dubois, E. AIS 7, 24 (1970).
129. Abeels, M., Dubois E., AIS 6, 10 (1969). /179
130. Adams, J.D., Nilan, B.A., Radiat. Res. 8, 111 (1958).
131. Adams, J.D., Nilan, B.A., Gunthard, H.M., Northwest Sci. 29, 101 (1955).
132. Adams, M.W., AIS 1, 7 (1964).
133. Amato F. d', In: "The Use of Induced Mutations in Plant Breedings," Report of Meeting of IAEA/FAO May 23-June 1, 1964, Rome.
134. Anand, R., AIS 3, 15 (1966).
135. Arnold, C.G., AIS 1, 17 (1964).
136. Arnold, C.G., AIS 2, 11 (1965).
137. Arnold, C .G, Cruse, D., Flora 155, 474 (1965).
138. Arnon, D., Amer. J. Bot. 25, 322 (1938).
139. Ashraf, J., AIS 7, 9 (1970).
140. Ashraf, J., AIS 7, 11 (1970a).
141. Ashraf, J., AIS 8, 11 (1971).
142. Ashraf, J., AIS 8, 11 (1971a).
143. Baetcke, K. P., Sparpow, A.H., Nauman, C.H., Schwemmer, S.S., Proc. Nat. Acad. Sci., USA, 58, 533 (1967).
144. Bailey, N., Statisticheskiye methody v biologii (Statistical methods in biology), Moscow, 1962.
145. Bailey, N., Matematika v biologii i meditsine (Mathematics in biology and medicine), Moscow, 1970.
146. Balkema, G.H., AIS 5, 59 (1968).
147. Barabas, Z., Redei, G.P. AIS 8, 7 (1971).
148. Barendse, G.W.M., In: Tuinbouwkundig Onderzoeh Jaars-verslag 1963, Abtr. The Hague, Ministry of Agriculture, 1963, p. 281.
149. Berg, B.I. van den, Feenstra, W.J., AIS 5, 27 (1968).
150. Bergbusch, V.L., Caldecott, R.S., Radiat. Res. 2, 207 (1963).
151. Berger, B., In: Arabidopsis Research. Rept. Internat. Sympos. Univ. Goettingen, April 21-24, 1965. G. Roebbelen, Ed. Suppl. I to AIS 19 (1965).
152. Berger, B., AIS 3, 13 (1966).
153. Berger, B. , Beitr. Biol. Pflanzen 45, 171 (1968).

154. Berger, B., AIS 5, 23 (1968a).
155. Berger, B., AIS 5, 24 (1968b).
156. Bhatia, C.R., Mutation Res. 5, 375 (1967).
157. Bhatia, C.R., AIS 4, 67 (1967a).
158. Bhatia, C.R., Narayanan, K.R., Genetics 52, 577 (1965).
159. Bhatia, C.R., Narayanan, K.R., AIS 2, 21 (1965a).
160. Bliss, F.A., Roebbelen, G., AIS 6, 4 (1969).
161. Boag, J.W., "The RBE of different ionizing radiations," NBS Rept. # 2946, Washington, 1953.
162. Boecher, T.W., Larsen, K., Kgl. Danske vid. selskal. Biol. med. 10, 1 (1958).
163. Boelpaepe, R.,de., Agricultura (Belg) 16, 48 (1968).
164. Bonotto, S., Experientia 22, 481 (1966).
165. Bonotto, S., Jacobs, M., AIS 5, 59 (1968).
166. Bonotto, S., Jacobs, M. AIS 9, 9 (1968).
167. Bora, K.C., Proc. 2nd Interna. Conf. Peaceful Uses Atom. Energy, Geneva, 1958 2, 22.
168. Bora, K.S., In: "Effects of Ionizing Radiations on Seeds," IAEA, Vienna, 1961 p. 345.
169. Bouharmont, J., In: Arabidopsis Research, Report. Internat. Symp. Univ. Goettingen, Suppl. 1 to AIS 31 (1965).
170. Bouharmont, J., In "Chromosomes Today," Proc. 1st Oxford Chromosome Conf. , N.Y., Plenum Press, 1966.
171. Bouharmont, J., AIS 4, 3 (1967).
172. Bouharmont, J., In: "Induced Mutations in Plants," Proc. Sympos. IAEA/FAO, IAEA, Vienna, 1969, p. 603.
173. Bowen, H.J.M., Abstr. Communs 2nd Internat. Congr. Radiat. Res., 5-11 Aug. 1962, Harrogate, 1963, p. 111.
174. Bridges, C.B., Brehme, K.S., The mutants of Drosophila melanogaster, Carnegie Inst. Publishers, 1944, p. 552.
175. Brock R.D., AIS 2, 6 (1965). /180
176. Brock, D.R., In: "Induced Mutations and Their Utilization" Erwin-Baur-Gedachtnis-Vorlesungen IV. -Abh. Dtsch. Akad. Wiss. Berlin, Kl, Med., 1967, 2, p. 263.
177. Brock, R.D., Radiat. Bot. 7, 193 (1967a).
178. Bronkers, F., Pollen et spores 5, 233 (1963).
179. Brown, J.A.M., Plant Physiol. Suppl. 37, 22 (1962).
180. Brown, J.A.M., Genetics 50, 237 (1964).
181. Brown, J.A.M., AIS 4, 29 (1967).
182. Brown, J.A.M., AIS 4, 30 (1967a).
183. Brown, J.A.M., AIS 6, 16 (1969).
184. Brown, J.A.M, Bhatia, C.R., Smith, H.M., In: Arabidopsis Research, "Report. Internat. Sympos. Univ. Goettingen, 1965, p. 171.
185. Brown, J.A.M., Klein, W.H., Plant Physiol. 47, 399 (1971).
186. Brown, J.A.M., Smith, H.H., Mutation Res. 1, 45 (1964).
187. Bucholz, J.T., Blakeslee, A.F., J. Heredity 21, 119 (1930).
188. Bucholz, J.T., Blakeslee, A.F., Genetics 21, 731 (1936).
189. Buerger, D., Roebbelen, G., AIS 7, 27 (1970).
190. Buggert, F., Roebbelen, G., AIS 7, 33 (1970).
191. Buiatti, M., Lorenzoni, C., Caryologia 16, 101 (1963).

192. Buzzati-Traverso, A.A., Cavalli, L.L. Theoria dell'urto ed unita biologiche elementari, Milan, Longanesi and Co., 1948.
193. Caldecott, R.S., Sciences 120, 809 (1954).
194. Caldecott, R.S., Proc. 2nd Internat. Congr. Peaceful Uses Atom. Energy, Geneva, 2, 260 (1958).
195. Caldecott, R.S., In: "Effects of Ionizing Radiations on Seeds," Sympos. IAEA, Vienna, 1961, p. 11.
196. Caldecott, R.S., Beard, B.H., Gardner, C.O. Genetics 39, 240 (1954).
197. Caldecott, R.S., Smith, L., Genetics 37, 136 (1952).
198. Campbell, R.C., Statistics for Biologists, Cambridge U. Press, 1967.
199. Cetl, I., In: Arabidopsis Research, Supp. I to AIS 46 (1965).
200. Cetl, I.,"Ueber die Korrektion der Mutationshaeufigkeit mittels ler Wahrscheindlichkeit des Auftretens von Doppelmutationen", In: Problemen der Pflanzengenetik, Olomouc, 1969, p. 45.
201. Cetl, I., Adamcova, J., Kucerova, M., AIS 3, 29 (1966).
202. Cetl, I., Dobrovolna, J., AIS 5, 15 (1968).
203. Cetl, I., Dobrovolna, J., Effmertova, E., AIS 2, 3 (1965).
204. Cetl, I., Dobrovolna, Effmertova, E., Biologia 18, 37 (1968).
205. Cetl, I., Dobrovolna, J., Effmertova, E., Biologia 22, 1 (1969).
206. Cetl, I., Relichova-Dobrovolna, J., Krskova, M., AIS 7, 35 (1970).
207. Clauss, H., Rauch, W.Z., Bot. 44, 437. (1956).
208. Contant, R.B., AIS 3, 34 (1966).
209. Contant, R.B., AIS 3, 35 (1966a).
210. Contant, R.B., AIS 5, 39 (1966b).
211. Contant, R.B., Dankert, R., AIS 5, 40 (1968).
212. Contant, R.B., Dankert, R., AIS 5, 43 (1968b).
213. Corcos, A.F., AIS 6, 15 (1969).
214. Corcos, A., AIS 7, 22 (1970).
215. Corcos, A., AIS 9, 36 (1972).
216. Corcos, A., Lewis, R., AIS 8, 35 (1971).
217. Corcos, A., Lewis, R., AIS 9, 18 (1972).
218. Corcos, A., Lewis, R., AIS 9, 9 (1972a).
219. Crovello, T.J., AIS 5, 7 (1968).
220. Daly, K., Radiat. Res. 12, 430 (1960).
221. Daly, K., Genetics 45, 983 (1960a). /181
222. Daly, K., Genetics 46, 861 (1961).
223. Dankert, R., Contant, R. B., AIS 5, 46 (1968).
224. Davidson, D., In: Radiation protection and recuperation Moscow, 1964, p. 182.
225. Davies, D.R., Radiat. Res. 19, 185 (1963).
226. Davies, D.R., Radiat. Res. 20, 726 (1963a).
227. Davies, D.R., Evans, H.G., In Advances in Radiation Biology vol. 2, N.Y., 1966, p. 243.
228. De Candolle, A. P., Prodromus systematis naturalis regni

vegetabilis, Paris-Geneva, 1825.
229. Devi, S.K., Prasad, K.N., Riley, E.F., Radiat. Bot. 4, 347 (1964).
230. Dierks, W.Z., Pflanzenzuecht 40, 67 (1958).
231. Dobrovolna, J., AIS 4, 6 (1967).
232. Dobrovolna, J., AIS 5, 17 (1968).
233. Donini, B., Sparrow, A.H., Schairer, L.A., Sparrow, R.C., Radiat. Res., 32, 692 (1967).
234. Effmertova, E., AIS 4, 8 (1967).
235. Effmertova, E., Cetl, I., AIS 3, 10 (1966).
236. Effmertova, E., Cetl, I., AIS 5, 16 (1968).
237. Ehrenberg, L., In: Voprosy radiobiologii [Questions on Radiobiology], Moscow, 1956, p. 453.
238. Ehrenberg, L., Anderson, G., Nature 173, 1086 (1954).
239. Ehrenberg, L., Gustafsson, A., Acta radiol. 41, 101 (1954).
240. Ehrenberg, L., Gustafsson, A., Lundquist, V., Nybom, N., Hereditas 39, 493 (1953).
241. Ehrenberg, L., Lundquist, V., Hereditas 43, 390 (1957).
242. Ehrenberg, L., Saeland, E., J. Nuclear Energy 1, 150, (1954).
243. Evans, H.J., Physiol. Med. Biol. 3, 411 (1959).
244. Evans, H.J., Sparrow, A.H., Brookhaven Sympos. Biol. 14 101 (1961).
245. Eyster, W.H., Bibliogr. genetica, 1934.
246. Feenstra, W.J., AIS 1, 23 (1964).
247. Feenstra, W.J., In: Arabidopsis Research, Suppl. 1 to AIS 113 (1965).
248. Feenstra, W.J., Ibid.
249. Feenstra, W.J., AIS 2, 24 (1965a).
250. Feenstra, W.J., AIS 2, 25 (1965b).
251. Feenstra, W.J., AIS 2, 33 (1965c).
252. Feenstra, W.J., AIS 2, 34 (1965d).
253. Feenstra, W.J., Genetics 35, 259 (1966).
254. Feenstra, W.J., AIS 4, 62 (1967).
255. Feenstra, W.J., Heyting, J., AIS 3, 18 (1966).
256. Fisher, R.A., The Design of Experiments, London, 1951.
257. Fisher, R.A., Statisticheskiye metody dlya issledovateley [Statistical methods for scientists], Moscow, 1958.
258. Freyn, J., Oesterr. Bot. Z. 39, 101 (1889).
259. Froese-Gertzen, E. E., Konzak, C. F., Nilan, R. A., Heiner, R. A., Radiat. Bot., 4, 61 (1964).
260. Frydenberg, O., Radiat. Bot. 3, 135 (1963).
261. Fujii, T., Japan. J. Genet. 39, 91 (1964).
262. Fujii, T., AIS 2, 17 (1965).
263. Fujii, T., AIS 3, 20 (1966).
264. Fujii, T., AIS 4, 59 (1967).
265. Fujii, T., AIS 5, 47 (1968).
266. Fujii, T., Japan. J. Genet. 44, 431 (1969).
267. Fujii, T., Ikenaga, M., Lyman, J. T., Radiat. Bot. 6, 297 (1966).
268. Fujii, T., Ikenaga, M., Lyman, J. T., Nature 213, 175 (1967).

269. Gaul, H., Flora 147, 207 (1959).
270. Gaul, H., Genetica agraria 12, 297 (1960).
271. Gaul, H., Radiat. Bot. 4, 155 (1964).
272. Gaul, H., In: "Induction of Mutations and the Mutation Process," Proc. Sympos. Praha, 27-29 Sept. 1963, 1965, p. 62
273. Gavazzi, P., Redei, G. P., AIS 8, 17 (1971). /182
274. Gichner, T., In: Arabidopsis Research, Rept. Int. Symps. Univ. Goettingen, 1965, p. 200.
275. Gichner, T., Ehrenberg, L., Wachtmeister, C.A., Hereditas 59, 253 (1968).
276. Gichner, T., Ehrenberg, L., Wachtmeister, C.A., AIS 5, 53 (1968a).
277. Gichner, T., Veleminsky, J., In: "Induction of Mutations and The Mutation Process," Praha, 27-29 Sept. 1963, 1965, p. 54.
278. Gichner, T., Veleminsky, J., Biol. plantarum (Praha) 7, 162 (1965a).
279. Gichner, T., Veleminsky, J., Mutation Res. 4, 207 (1967).
280. Gichner, T., Veleminsky, J., AIS 5, 52 (1968).
281. Gichner, T., Veleminsky, J., "The mutagenic activity of nitrosamides and nitrosamines on a plant--Arabidopsis thaliana," Environmental Mutagenic Scoiety Newsletter 3, (1970).
282. Gichner, T., Veleminsky, J., AIS 8, 30 (1971).
283. Gomez-Campo, C., Delgado, L., Radiat. Bot. 4, 479 (1964).
284. Gray, L.H., Scholes, M.E., Brit. J. Radiol. 24, 82 (1951).
285. Greenblatt, C.L., Internat. J. Radiat. Biol. 4 (1961).
286. Gregory, F. G., Hussey, G.G., Proc. Linnean Soc. London, 164, 137 (1953).
287. Grover, N.S., Byrne, O. R., AIS 9, 10 (1972).
288. Guilleminot, H.C., Compt. rend. Soc. biol. 1, 309 (1910).
289. Gustafsson, A., Hereditas 22, 281 (1937).
290. Gustafsson, A., Lunds Univ. Arsskrift. N.F. 36 (1940).
291. Gustafsson, A., Lundquist, U., Ekberg, I., Hereditas 56, 200 (1967).
292. Haberer, G., Eichhorn, K., Abel, H., Ivanov, V., Studia biophysica (Berlin) 6, 113 (1968).
293. Haberer, G., Regel, K., Studia biophysica (Berlin) 17, 29 (1969).
294. Haerer, L., Beitr. Biol. Pflanzen 28 (1951).
295. Hall, E.J., Laitha, L.G., Clowes, A.L., Abstr. Communs 2nd Internat. Congr. Radiat. Res., 5-11 Aug. 1962, Harrogate, p. 30.
296. Harle, J.R., AIS 9, 34 (1972).
297. Hayek, A.V., Flora von Steiermark. Spezieller Tell. Bd. 1. Berlin. Beih. Bot. Zbl. 27, 127 (1908).
298. Heaslip, M.B., IN: "Radioecology (1st Nat. Symp. Colorado Univ., 1961)", 1963, p. 301.
299. Hirono, Y., Genetics 50, 255 (1964).

300. Hirono, Y., Thesis, Univ. Missouri, 1964a.
301. Hirono, Y., AIS 2, 9 (1965).
302. Hirono, Y., Combatti, N.C., Smith, H.H., AIS 4, 61 (1967).
303. Hirono, Y., Redei, G. P. In: "Genetics Today".-- Proc. XI Int. Congr. Genetics, The Hague, Sept. 1963 1, p. 15.
304. Hirono, Y., Redei, G. P. Nature 197, 1324 (1963a).
305. Hirono, Y., Redei, G. P. , AIS 1, 21 (1964).
306. Hirono, Y., Redei, G. P. Genetics,51, 519 (1965).
307. Hirono, Y., Redei, G. P. In: Arabidopsis Research, "Rept. Int. Smp."Suppl. 1 to AIS 85 (1965a).
308. Hirono, Y., Smith, H.H., Proc. XVI Int. Cong. Genet., Tokyo, Aug. 19-28, 1968, 1, p. 117.
309. Hirono, Y., Smith, H.H., AIS 5, 32 (1968a).
310. Hirono, Y., Smith, H.H., Genetics 61, 191 (1969).
311. Hirono, Y., Smith, HH., Lyman, J., AIS 5, 47 (1968).
312. Hirono, Y., SMith, H. H., Lyman, J. T., Thompson, K. H., Baum, J. W., Radiat. Res. 44, 204 (1970).
313. Howell, M.J., AIS 6, 13 (1969).
314. Hussein, H. A. S., Ph. D. Thesis, Wageningen, 1968.
315. Hussein, H. A. S., AIS 6, 18 (1969).
316. Hussein, H. A. S., AIS 7, 9 (1970).
317. Hussein, H. A. S., Veen J. H. van der., AIS 2, 6 (1965).
318. Hussein, H. A. S., Veen J. H. van der, AIS 5, 30 (1968).
319. Jacobs, M. Lejeunia 25, 1 (1964). /183
320. Jacobs, M., Bull. Soc. roy. Bot. Belgique 98, 43 (1964a).
321. Jacobs, M., AIS 1, 22 (1964b).
322. Jacobs, M., AIS 1, 36 (1964c).
323. Jacobs, M., Bull. Acad. roy. Belgique. Cl. sci. ser. 5, 51, 725 (1965).
324. Jacobs, M., In: Arabidopsis Research; Suppl. 1 to AIS 106 (1965a).
325. Jacobs, M., AIS 2, 20 (1965b).
326. Jacobs, M., AIS 2, 27 (1965c).
327. Jacobs, M., Bull. Soc. roy. bot. Belg. 100, 259 (1967).
328. Jacobs, M., AIS 4, 40 (1967a).
329. Jacobs, M., Bull. Soc. roy. bot. Belg. 102, 1 (1968).
330. Jacobs, M., Bull. Soc. roy. bot. Belg. 102, 2 (1969).
331. Jacobs, M., Mutation Res. 7, 51 (1969a).
332. Jacobs, M., Radiat. Bot. 9, 251 (1969b).
333. Jacobs, M., Mutation Res.7, 51 (1969c).
334. Jacobs, M., Bonotto, S., AIS 4, 49 (1967).
335. Jacobs, M., Bonotto, S., AIS 5, 34 (1968).
336. Jacobs, M., Schwind, F., AIS 9, 11 (1972).
337. James, T., AIS 7, 22 (1970).
338. Jaretzki, R., Jahrb. wiss. Bot. 68, 1 (1928).
339. Jaretzki, R., Jahrb. wiss. Bot. 76, 485 (1932).
340. Jones, M.E., AIS 5, 11 (1968).
341. Jones, M.E., Heredity 27, 39 (1971).
342. Jones, M.E., Heredity 27, 51 (1971a).
343. Jones, M.E., Heredity 27, 59 (1971b).

344. Karbe, C., Roebbelen, G., AIS 5, 13 (1963).
345. Karlovska, V., AIS 7, 4 (1970).
346. Kawai, F., Sato, H., In: "Mutations in Plant Breedings," Sympos. IAEA, Vienna, 1966, p. 151.
347. Kwai, T., IN: "Induced Mutations in Plants," Proc. Symp. IAEA/FAO, IAEA, Vienna, 1969, p. 137.
348. Kitamura, S., Flora of Afghanistan, Kyoto, 1960.
349. Koernicke, M., Ber. dtsch. bot. Ges. 22, 148 (1904).
350. Koernicke, M., Ber. dtsch. bot. Ges. 23, 324 (1905).
351. Koernicke, M., Jahrb. wiss. Bot. 55, 416 (1915).
352. Koernicke, M., Z. phys. und diaetat. Therapie 6, 363 (1916)
353. Koernicke, M., Fortschr. Geb. Roentgenstrahlen 27, 661 (1919).
354. Kondo, S., In: "Mechanism of the Dose Rate Effect of Radiation at the Genetic and Cellular Level," Tokyo, Genet. Soc. Japan, 1965, p. 97.
355. Konzak, C.F., Science 122, 3161 (1955).
356. Konzak, C.F., Caldecott, R.S., Delihas, N., Curtis, H. I., Radiat. Res. 7, 326 (1957).
357. Konzak, C.F., Curtis, H. I., Delihas, N., Nilan, R.A., Canad. J. Genet. Cytol. 2, 129 (1960).
358. Konzak, C.F., Nilan, R.A., Legault, R.R., Heiner, R.E., In: "Action of Ionizing Radiations on Plant Seeds," Proc. Sympos. Karlsruhe, 1961. p. 155.
359. Kranz, A.R., AIS 5, 56 (1968).
360. Kranz, Z. R., AIS 7, 28 (1970).
361. Kranz, A. R., Theoret. and Appl. Genetics 41, 45 (1971).
362. Kranz, A. R., Theoret. and Appl. Genetics, 41, 91 (1971a).
363. Kranz, A.R., Theoret. and App. Genetics 41, 191 (1971b).
364. Kranz, A. R., AIS 9, 19 (1972).
365. Kribben, F. L., Naturwiss. Rundsch. 17, 139 (1964).
366. Kribben, F. J., In: Arabidopsis Research; Supp. 1 to AIS 26 (1965).
367. Kucera, J., AIS 2, 4 (1965).
368. Kucera, J., In: "Mechanism of Mutation and Inducing Factors," /184 Prague, Academia, 1966, p. 313.
369. Kucera, J., AIS 3, 32 (1966a).
370. Kucera, J., Cetl, I., Publ. Fac. Sci. Univ. Purkyne, Brno 479, 1(1967).
371. Kuo, S.C., Redei, G. P., AIS 8, 25(1971).
372. Laan, P. H., van der, Oostindier-Braaksma, F. J., Feenstra, W. J., AIS 8,22(1971).
373. Laibach, F., Beih. Bot. Zbl., I Abt. 22, 191(1907).
374. Laibach, F., Natur. und Volk 70, 55 (1940).
375. Laibach, F., Bot. Arch., 44, 439(1943).
376. Laibach, F., Naturwissenschaften 31, 246 (1943a).
377. Laibach, F., Ber. Dtsch. bot. Ges. 62, 27 (1949).
378. Laibach, F., Beitr. Biol. Pflanzen 28, 173(1951).
379. Laibach, F., Planta 51, 148(1958).
380. Laibach, F., Zenker, A., Planta 43, 250(1954).
381. Lamprecht, H., Agrihort. Genet. 18, 135(1960).

382. Lamprecht, H., In: Arabidopsis Research; Suppl. 1 to AIS 208 (1965).
383. Langridge, J., Nature 176, 260(1955).
384. Langridge, J., Austral. J. Biol. Sci. 10, 243(1957).
385. Langridge, J., Austral. J. Biol. Sci. 11, 58 (1958).
386. Langridge, J., Austral. J. Biol. Sci. 11, 457(1958a).
387. Langridge, J., Amer. Naturalist 96, 5(1962).
388. Langridge, J., In:Environ. Contr. of Plant Growth, London, 1963.
389. Langridge, J., Annual Rev. Plant. Phys. 14, 441 (1963a).
390. Langridge, J.,Austral. J. Biol. Sci. 18, 311 (1965).
391. Langridge, J., Griffing, B., Austral. J. Biol. Sci. 12,117(1959).
392. Lawrence, C.W., AIS 3, 36(1966).
393. Lawrence, M. J., Snape, J.W., AIS 8, 6(1971).
394. Lea, D.E., Action of Radiation of Living Cells, Camb.U., 1946.
395. Lea, D.E., Deystviye radiatsii na zhivyye kletki [Action of radiation on living cells], Moscow, 1963.
396. Ledoux, L., Jacobs, M., AIS 6, 5(1969).
397. LeeChen, S., Buerger, D., AIS 4, 4(1967).
398. Lee-Chen, S., Steinitz-Sears, L. M., Canad. J. Genet. and Cytol. 9, 381 (1964).
399. Lee-Chen, S., Steinitz-Sears, L. M., AIS 6, 22(1969).
400. Li, S., AIS 4, 5(1967).
401. Li, S. L., Redei, G. P., Trans. Missouri Acad. Sci. 1, 78 (1967).
402. Li, S. L., Redei, G. P., AIS 5, 28(1968).
403. Li, S. L., Redei, G. P., AIS 5, 58(1968a).
404. Li, S. L., Redei, G. P., Radiat. Bot. 9, 125 (1969).
405. Li, S. L., Redei, G. P., Plant Physiol. 44, 225(1969a).
406. Li, S. L., Redei, G. P., Genetics,62, 281(1969b).
407. Li, S. L., Redei, G. P., Theoret. and Appl. Genet. 39, 68 (1969c).
408. Li, S. L., Redei, G. P., Gowans, C. S., Molec. and Gen. Genetics 100, 77 (1967).
409. Lindegren, C.C., Lindegren, G., Indian Phytopathol. 4, 11(1951).
410. Lindsley, D.L., Grell, E.H., CarnegieInst. Washington Publ. 1968, 627.
411. Linnaeus, K., Genera plantarum, Leiden, 1737.
412. Loeve, A., Loeve, D., Opera Botanica (Soc. Bot. Lundensi) 5, 1(1961).
413. Loewenberg, J.R., AIS 2, 34(1965).
414. Loewenberg, J.R., Thompson, P. J., AIS 4, 68(1967).
415. Mabuchi, T., AIS 9, 35 (1972).
416. McKelvie, A.D., Genet. agrar.(Pavia) 14, 197(1961).
417. McKelvie, A.D., Radiat. Bot. 1, 233 (1962).
418. McKelvie, AD., Radiat. Bot. 3, 105(1963).
419. McKelvie, A.D., IN: Arabidopsis Research, Suppl. to AIS 79, (1965).
420. Manton, I., Ann. Bot. 46, 509(1932).
421. Mather, K., The Measurement of Linkage in Heredity, N.Y., 1957/185

422. Miksche, J. P., Abstr. Commun, 2nd Internat. Congr. Radiat. Res., 5-11 Aug. 1962, Harrogate, 1963, p. 112.
423. Miksche, J. P., Brown, J. A. M., Amer. J. Bot. 50, 616(1963).
424. Miksche, J. P., Brown, J. A. M., Amer. J. Bot. 52, 533(1965).
425. Mostafa, I. V., Naturwissenschaften 52, 108(1965).
426. Mueller, A.J., Kulturpflanze 9, 364(1961).
427. Mueller, A.J., Naturwissenschaften 48, 579 (1961a).
428. Mueller, A.J., Biol. Zbl. 82, 133(1963).
429. Mueller, A.J., Kulturpflanze 12, 237(1964).
430. Mueller, A. J., Zuechter 34, 102 (1964a).
431. Mueller, A.J., In: "Induction of Mutations and the Mutation Process," 1965. Proc. Sympos. Praha, 27-29 Sept. 1963, 1965, p. 46.
432. Mueller, A.J., Mutation Res. 2, 425 (1965a).
433. Mueller, A. J., Kulturpflanze 13, 163 (1965b).
434. Mueller, A.J., Wissenschaften 52, 213(1965c).
435. Mueller, A.J., Biol. Zbl. 84, 759 (1965d).
436. Mueller, A.J., In: Arabidopsis Research, Suppl. 1 to AIS 152 (1965e).
437. Mueller, A.J., In: Arabidopsis Research, Rep. Int. Symp. Univ. Goettingen, 1965f, p. 192.
438. Mueller, A.J., AIS 2, 19(1965g).
439. Mueller, A.J., AIS 2, 22(1965h).
440. Mueller, A.J., Zuechter 36, 201(1966).
441. Mueller, A.J., AIS 3, 22(1966a).
442. Mueller, A.J., AIS 3, 22(1966b).
443. Mueller, A.J., AIS 3, 23(1966c).
444. Mueller, A.J., AIS 3, 24(1966d).
445. Mueller, A.J., AIS 3, 24(1966e).
446. Mueller, A.J., AIS 3, 24 (1966f).
447. Mueller, A.J., In: Induced Mutations and Their Utilization, Berlin, Akademie Verlag, 1967, 2, p. 89.
448. Mueller, A.J., Biol. Zbl., Suppl. 86, 89(1967a).
449. Mueller, A.J., AIS 4, 42(1967b).
450. Mueller, A.J., AIS 4, 43(1967c).
451. Mueller, A. J., AIS 5, 53(1968).
452. Mueller, A. J., AIS 6, 28(1969).
453. Mueller, A. J., AIS 6, 28(1969a).
454. Mueller, A.J., AIS 8, 33(1971).
455. Mueller, A.J., Gichner, T., Nature 201, 1149 (1964).
456. Mueller, A.J., Heidecker, V., AIS 5, 54(1968).
457. Napp-Zinn, K., Kosmos 49, 65(1953).
458. Napp-Zinn, K., "Vernalisation und Blattbildung," Rapp. et. Communs VIII Congr. internat. Bot. 11/12, 287(1954).
459. Napp-Zinn, K., Naturwissenschaften 42, 650 (1955).
460. Napp-Zinn, K., Mat. und Naturwiss. Unterr. 13, 154(1960).
461. Napp-Zinn, K., Beitr. Biol. Pflanzen 38, 161(1963).
462. Napp-Zinn, K., In: Beitraege zur Phytologie. Festsch. H. Walter, K. Kreeb, Ed., Hrsg. 1964, p. 33.
463. Napp-Zinn, K., In: Arabidopsis Research, Supp. 1 to AIS 56(1965).

464. Napp-Zinn, K., Berset, D., AIS 3, 37(1966).
465. Neales, T.F., New Phytologist 67, 159(1968).
466. Neary, G.J., Savage, J.R.K., Evans, H.J., Whittle, J., Internat. J. Radiat. Biol. 6, 127 (1963).
467. Neary, G.J., Tonkinson, S.M., Williamson, F. S., Internat. J. Radiat. Biol. 1, 201 (1959).
468. Nilan, R. A., Genetika 3, 3(1967).
469. Nilan, R. A., Konzak, C.T., Legault, R. K., Harle, J.R., In: "Effects of Ionizing Radiations On Seeds," Proc. Symp. IAEA, Vienna, 1961, p. 139.
470. Oostinider-Braaksma, F. J., AIS 7, 24(1970). /186
471. Oostinider-Braaksma, F. J., Feenstra, W. J., AIS 9, 9(1972).
472. Parsa, A., Flora de l'Iran, v. 1, Teheran, 1951.
473. Petrova, J., Beih. Bot. Zbl. 6, Abt. A, 399 (1942).
474. Postlethwait, S.N., Enochs, N.J., Plant. Sci. Bull. 13(2), 1 (1967).
475. Prantl, K., Die natuerlichen Pflanzenfamilien, Bd. III, A. Engler and K. Prantl, Eds. 1891, III, p. 2.
476. Ratcliffe, D. J. Ecol. 49, 187(1961).
477. Ratcliffe, D. in: Arabidopsis Research, Rep. Internat. Symp. Univ. Goettingen, April 21-24, 1965, p. 37.
478. Read, J., Radiation biology of Vicia faba in relation to the general problem, Oxford, 1959.
479. Redei, G. P., Genetics 45, 1007 (1960).
480. Redei, G. P., Genetics 47, 979(1962).
481. Redei, G. P., Z. Vererbungslehre 93, 164 (1962a).
482. Redei, G. P., Genetics 47, 443(1962b).
483. Redei, G. P. Science 139, 767 (1963).
484. Redei, G. P., Genetics 50, 278(1964).
485. Redei, G. P., AIS 1, 10(1964a).
486. Redei, G. P., AIS 1, 11(1964b).
487. Redei, G. P., AIS 1, 14(1964c).
488. Redei, G. P., AIS 1, 25(1964d).
489. Redei, G. P., Genetics 51, 857 (1965).
490. Redei, G. P., Amer. J. Bot. 52, 834 (1965a).
491. Redei, G. P., AIS 4, 19(1967).
492. Redei, G. P., AIS 4, 64(1967a).
493. Redei, G. P., Experientia 23, 584(1967b).
494. Redei, G. P., AIS 5, 5(1968).
495. Redei, G. P., Bibliographia Genetica 21, 1(1969).
496. Redei, G. P., AIS 7, 3(1970).
497. Redei, G. P., AIS 9, 42(1972).
498. Redei, G. P., AIS 9, 41(1972a).
499. Redei, G. P., Barabas, Z., AIS 8, 27(1971).
500. Redei, G. PL, Hirono, Y., AIS 1, 9(1964).
501. Redei, G. P., Kuo, S.C., AIS 8, 22(1971).
502. Redei, G. P., Li, S. L., Genetics 61, 453(1969).
503. Redei, G. P., Perry, C. M., AIS 8, 34(1971).
504. Redei, G. P., Steinitz-Sears, L. M., Genetics 46, 892(1961).
505. Reinholz, E., Naturwissenschaften 34, 26(1947).

506. Reinholz, E., Fiat. Report. 1006 (1947a).
507. Reinholz, E., Strahlentherapie 95, 131 (1954).
508. Reinholz, E., Strahlentherapie 109, 537 (1959).
509. Reinholz, E., In: Max-Plank-Institut fuer Biophysik, 1937-1962, Frankfurt am Main, 1962, p. 88.
510. Reinholz, E., In: Arabidopsis Research, Rep. Int. Symp. Univ. Goettingen, Supp. 1 to AIS 9,(1965).
511. Reinholz, E., AIS 3, 19(1966).
512. Reinholz, E., AIS 9, 37(1972).
513. Reinholz, E., AIS 9, 27(1972a).
514. Reinholz, E., AIS 9, 38(1972b).
515. Relichova, J., AIS 9, 28(1972).
516. Rijven, A. H. G. C., Austral. J. Biol. Sci. 9, 511 (1956).
517. Roebbelen, G. Z. indukt. Abstammungs- und Vererbungslehre, 88, 189 (1957).
518. Roebbelen, G., Naturwissenschaften 44, 288 (1957a).
519. Roebbelen, G., Z. Naturforsc.13b, 14(1958).
520. Roebbelen, G., Vererbungslehre 90, 503(1959).
521. Roebbelen, G., Ber. Dtsch. bot. Ges. 73, 41(1960).
522. Roebbelen, G. Vererbungslehre 93, 25(1962).
523. Roebbelen, G., Z. Vererbungslehre 93, 127(1962a).
524. Roebbelen, G., Z. Vererbungslehre 93, 256(1962b). /187
525. Roebbelen, G. , Naturwissenschaften 49, 65(1962c).
526. Roebbelen, G., Ber. Dtsch. bot. Ges. 76, 416 (1964).
527. Roebbelen, G., AIS 1, 12(1964a).
528. Roebbelen, G., AIS 1, 20 (1964b).
529. Roebbelen, G., AIS 1, 22(1964c).
530. Roebbelen, G., In:"Induction of Mutations and the Mutation Process", Proc. Sympos. Praha, 27-29 Sept. 1963, 1965, p. 42.
531. Roebbelen, G., Beitr. Biol. Pflanzen 41, 323 (1965a).
532. Roebbelen, G, In: Arabidopsis Research, Suppl. 1 to AIS 100 (1965b).
533. Roebbelen, G., In: Arabidopsis Research; Supp. 1 to AIS 138 (1965c).
534. Roebbelen, G., AIS 2, 12(1965d).
535. Roebbelen, G., AIS 2, 36(1965e).
536. Roebbelen, G., In: "Mechanism of Mutation and Inducing Factors," Prague, Academia, 1966, p. 309.
537. Roebbelen, G., Genetics 54, 357 (1966a).
538. Roebbelen, G., Pflanzenphysiol. 55, 387 (1966b).
539. Roebbelen, G., Planta 69, 1(1966c).
540. Roebbelen, G., AIS 3, 7(1966d).
541. Roebbelen, G., AIS 5, 29(1968).
542. Roebbelen, G., AIS 8, 7(1971).
543. Roebbelen, G., AIS 9, 21 (1972).
544. Roebbelen, G., Kribben, F.J., AIS 3, 16(1966).
545. Roebbelen, G., Wehrmeyer, W., Planta 65, 105(1965).
546. Santos, I. S., In: "The Use of Induced Mutations in Plant Breedings," Proc. FAO/IAEA Techn. Meeting, Rome, 1965, p. 263.
547. Schober, A., Ber. Dtsch. bot. Ges. 14, 108(1896).

548. Scholz, H., Willdenowia 3, 137(1962).
549. Schulz, O. E., Die natuerlichen Pflanzenfamilien, Bd. 17b, Leipzig, 1936.
550. Seyffert, W. Z., Pflanzenzuecht 42, 356 (1960).
551. Smith, H.H., Bateman, J. L., Quastler, H., Rossi, H. H., In: "Biological Effects of Neutron and Proton Irradiations," v. II, Vienna, IAEA, 1964, p. 233.
552. Smith, H. H., Combatti, N.C., Rossi, H.H., In: "Neutron Irradiation of Seeds II," Vienna, IAEA, 1968, p. 3.
553. Smith, H.H., Hirono, Y., Conklin, M.E., Lyman, J.T., In: "Induced Mutations in Plants," Proc. Sympos. IAEA/FAO, Vienna, 1969, p. 231.
554. Smith, H.H., Joshua, D.C., Combatti, N.C., Thompson, K.H., In: "Symposium on Neutrons In Radiobiology," US AEC Conf. 691106 (TID--4500) Oak Ridge, Tenn., 1970, p. 173.
555. Smith, L., Caldecott, R.S., J. Heredity 34, 173 (1948).
556. Snape, J.W., Lawrence, M.J. Heredity 27, 299 (1971).
557. Sobels, F., Proc. XII Internat. Cong. Genetics 3, 205 (1969).
558. Sparrow, A.H., "The role of the cell nucleus in determining radiosensitivity," Brookhaven Lecture Series 17, BNL, 766(T-287), 1962.
559. Sparrow, A. H., In: Cellular Radiation Biology, Baltimore 1965, p. 199.
560. Sparrow, A.H., Baetcke, K. P., Shaver, D. L., Pond, V., Genetics 59, 68 (1967).
561. Sparrow, A.H., Christensen, E., Science 118, 697 (1953).
562. Sparrow, A.H., Cuany, R. L., Miksche, J., Radiat. Bot. 1, 10 (1961).
563. Sparrow, A.H., Evans, H.J., Brookhaven Sympos. Biol. 14, 76 (1961).
564. Sparrow, A.H., Sparrow, R.C., Thompson, K.H., Schairer, L.A., Radiat. Bot., 5(Suppl.), 101 (1965).
565. Stadler, L.J., Proc. Nat. Acad. Sci. USA 14, 69(1928).
566. Stadler, L. J., Heredity 21, 3(1930).
567. Stadler, L. J., Genetics 1, 274 (1932).
568. Stadler, L. J., Science 120, 811 (1954).
569. Stadler, L. J., Roman, H., Genetics 33, 273(1948).
570. Steinitz-Sears, L. M., Amer. J. Bot. 49, 663 (1962).
571. Steinitz-Sears, L. M., Genetics 48, 483 (1963).
572. Steinitz-Sears, L. M., AIS 1, 14(1964). /188
573. Steinitz-Sears, L. M., AIS 3, 36(1966).
574. Steinitz-Sears, L. M., Lee-Chen, S., AIS 5, 55(1968).
575. Steinitz-Sears, L. M., Lee-Chen, S., Canad. J. Genet. Cytol. 12, 217 (1970).
576. Svachulova, J., Photosynthetice 5(3), 249(1971).
577. Tauboeck, K., Bot. Arch. 44, 52 (1943).
578. Thoday, J. M., Brit. Radiol. 27, 572 (1951).
579. Trojan, R., AIS 8, 5(1971).
580. Tsuboi, M., Yanagishima, N., Plant and Cell Res. 12, 813(1971).
581. Vandendries, R., La Cellule 25, 412 (1909)
582. Vaughan, J. G., Nature 169, 458 (1952).

583. Vaughan, J. G., J. Linnean Soc. London, Bot. 55, 279 (1955).
584. Vaughan, J. G., Jones, F. R., Nature 171, 751 (1953).
585. Veen J. H. van der, In: Arabidopsis Research, Rep. Int. Symp. Univ. Goettingen. Suppl. 1 to AIS 62(1965).
586. Veen, J. H. van der AIS 2, 5(1965a).
587. Veen, J. H., van der, AIS 2, 31 (1965b).
588. Veen, J. H. van der, Genetics 38, 61(1967).
589. Veen, J. H. van der, AIS 4, 45 (1967a).
590. Veen, J. H. van der, AIS 4, 63 (1967b).
591. Veen, J. H. van der, AIS 5, 49(1968).
592. Veen, J. H. van der, Gerlach, M., AIS 2, 14(1965).
593. Veen, J. H. van der, Heemert, C. van, AIS 5, 50(1968).
594. Veen, J. H. van der, Jong, A. W. de, AIS 6, 21(1969).
595. Veen, J. H. van der, Wirtz, R., Euphytica 17, 371 (1968).
596. Veleminsky, J., Gichner, T., AIS 1, 34(1964).
597. Veleminsky, J., Gichner, Mutation Res. 5, 429(1968).
598. Veleminsky, J., Gichner, T., AIS 5, 52(1968a).
599. Veleminsky, J., Gichner, T., AIS 7, 26(1970).
600. Veleminsky, J., Gichner, T., Pokorny, V., Biol. plantarum (Praha) 7, 325 (1965).
601. Veleminsky, J., Gichner, T., Pokorny, V., Svachulova, J., AIS 1, 18(1964).
602. Veleminsky, J., Pokorny, V., Gichner, T., AIS 4, 48(1967).
603. Veleminsky, J., Pokorny, V., Gichner, T., Biol. plantarum (Praha) 10, 85(1968).
604. Veleminsky, J., Roebbelen, G., In: Arabidopsis Research, Rep. Int. Symp. Univ. Goettingen. Suppl. 1 to AIS 131(1965).
605. Veleminsky, J., Roebbelen, G., Planta 68, 15(1966).
606. Weiland, U., Mueller, A. J., AIS 8, 34(1971).
607. Weber, E. Mathematische Grundlagen der Genetik. Jena, 1967.
608. Wehrmeyer, E., Roebbelen, G., Planta 64, 312(1965).
609. Weiland, U., Mueller, A. J., AIS 9, 7(1972).
610. Westerman, J. M., Heredity 26, 93(1971).
611. Westerman, J. M., Heredity 26, 373(1971a).
612. Westerman, J. M., Heredity 26, 383 (1971b).
613. Westerman, J. M., Lawrence, M. J., Heredity 25, 609(1970).
614. Wibaut, C. C. r. Acad. sci. Paris 263, 343(1966).
615. Winge, O. La Cellule 35, 305(1925).
616. Wolf, S., Radiat. Res. 12, 484(1960).
617. Wolf, S., In: Cellular Radiation Biology, Baltimore, 1965, p. 167.
618. Wricke, G. Z. indukt. Abstammungs- und Vererbungslehre 87, 47(1955).
619. Yagyu, P., Morris, K., Genetics 42, 222 (1957).
620. Yokoyama, K., Jones, W.H., Plant Physiol. 40, LXXVII(1965).
621. Ziebur, N. K., AIS 2, 34(1965).
622. Zimmer, K. G., Ehrenberg, L., Ehrenberg, A., Strahlentherapie 103, 1(1957).
623. Zirkle, R. E., Amer. J. Cancer, 23, 558(1935).
624. Zirkle, R. E., J. Cell. and Compar. Physiol. 16, 221(1940).
625. Zirkle, R. E., In: Radiation Biology, vol. 1, New York, 1954, p. 315.